NC
267
U5226
1980
pt.2B

NATIONAL CANCER INSTITUTE

ANNUAL REPORT

October 1, 1979 through September 30, 1980

Part II-B

DIVISION OF CANCER BIOLOGY & DIAGNOSIS

Library
National Institutes of Health
Bethesda, Maryland 20014

DIVISION OF CANCER BIOLOGY AND DIAGNOSIS

ANNUAL REPORT

October 1, 1979 through September 30, 1980

TABLE OF CONTENTS

	Page
EXTRAMURAL RESEARCH PROGRAM	
ASSOCIATE DIRECTOR	
Summary Report	1
Tumor Biology Program	
Summary Report	5
Grants	17
Immunology Program	
Summary Report	47
Grants	59
Contracts	9[illegible]
Breast Cancer Program Coordination Branch	
Summary Report	151
Grants	161
Contracts	173
Cancer Diagnosis Research Program	
Summary Report	251
Grants	263
Contracts	271
Immunology Support Contracts	339
Biology Support Contracts	351

97
.626
980
t.28

EXTRAMURAL RESEARCH PROGRAM

DIVISION OF CANCER BIOLOGY AND DIAGNOSIS

This year marks the first complete year for the newly created Extramural Research Program. It consists of four program areas organized in three Branches: Diagnosis, Cancer Biology and Breast Cancer Program Coordinating Branch. After the reorganizational moves were completed, the programs began to function in an autonomous fashion, interacting in areas of overlapping interests but shaping their individual programs slone, with the help of the two Advisory Committees: Diagnosis Research Advisory Group and Breast Cancer Task Force. A Steering Committee consisting of four Program Directors, Director and Administrative Officer, DCBD, and Associate Director for Extramural Research Program served as a forum for coordination of the entire Extramural Program. Except for the Tumor Biology Program which is entirely grant based, both contracts and grants have been utilized in funding various projects. The Contract Supported Research Program continued the established trend of the conversion to grants.

Next year the entire Contract Program will be at the level of about $14,000,000, a more than $20,000,000 conversion in three years from contracts to grants.

The Tumor Biology Program supports a broad spectrum of basic biological and biochemical research in the pursuit of one overriding goal, defining properties of cancer cells and tumors that uniquely distinguish them from normal, healthy cells and tissues. There are three major areas of investigation which correspond to different theories of how to control the development and progression of neoplastic disease. The first is understanding the basic biochemical mechanisms involved in growth control, whether these involve particular external signals that initiate the process of cell division or particular internal molecules more directly responsible for DNA replication and metabolism. The second is studying changes that occur at the molecular level which lead to cancer cell invasion. The invasion behavior of cancer cells is a prerequisite to malignancy, or the ability of tumors to invade surrounding tissues, escape normal host defense mechanisms and become established at multiple secondary metastatic sites. The third is to develop detailed biological and biochemical information about the processes which induce cancer cell differentiation. There is good reason to believe that many kinds of cancers will respond to external stimuli and differentiate. If the genetic program of an actively growing cancer could be changed to one of terminal differentiation, then the malignant tumor could be rendered harmless.

The Immunology Program is composed of three sections with focus on Molecular Immunology, Cellular Immunology and Clinical Immunology. Support is provided for a spectrum of research which reflects the need for more information on basic aspects of the immune system as well as the importance of translating that information to studies on animal tumors and ultimately to the clinic and human cancer. The Program supports investigations on the effects of the immune system upon the initiation, development, growth, spread and control of tumors.

Some of the more specific areas include the following:

The synthesis, structure and function of antibodies and humoral factors other than antibody which participate in, activate and/or regulate the immune response to tumors. This would include complement, interferon, lymphokines, lymphoid cell growth factors, helper factors, suppressor factors, etc., as they are involved in immune responses to tumors;

The immunobiology of malignancies of the immune system (lymphomas and leukemias) including studies of immunologic markers for the classification and characterization of neoplastic cells and their normal counterparts;

The immunobiology of lymphocytes, monocytes and macrophages which participate in anti-tumor responses including their development, heterogeneity, interactions and functions;

The identification, isolation and characterization of cell surface determinants of lymphocytes and macrophages which are involved in the responses of these cells to tumors;

Immune surveillance against the development of tumors of various origins by all immune mechanisms (e.g., T cell immunity, macrophage reactivity, natural killer cell activity).

Bone marrow transplantation in man and animals as a treatment for cancer when the emphasis is on immunologic problems such as immune reconstitution, sensitization and graft versus host disease.

Immunotherapy including pre-clinical and clinical protocols where the main emphasis is upon the study of immune parameters, immune mechanisms and other immunologic concerns rather than upon a therapeutic result. Included are studies on specific and non-specific stimulation of the immune system using natural and synthetic agents.

The Cancer Diagnosis Research Program emphasizes research in early detection, diagnosis and monitoring of cancer as well as the application of this knowledge to the appropriate populations for clinical evaluation. It is concerned with improvement of existing techniques as well as development of new tests and procedures. Research in detection and diagnosis attempts to recognize and localize cancer in the earliest possible stage. Such recognition should improve the chances for control of the disease and increase the duration and quality of life. The program seeks to establish modalities for early recognition in general as well as an improved definition of early lesions in specific body sites. Research in cancer diagnosis also includes the development of knowledge, methods and techniques to establish the presence, location and extent of cancer and precancerous conditions and prognostic indications. In addition, it seeks to evaluate the clinical efficacy of these approaches. It should be recognized that, in the earliest stages of development, research may not be site oriented. Also in such early stages, research efforts may be involved with animal model systems as well as with human disease.

Currently the program covers eight disciplines: biochemistry, immunodiagnosis, cytology, pathology, radiological imaging, non-radiological imaging, nuclear medicine and multiple disciplines. Some research on diagnosis is carried out in two other Divisions: Treatment and Cause and Prevention, as an integral part of their program, where the major emphasis is on prognostic and monitoring activities or preventive aspects.

The oldest Extramural Program in DCBD, and the oldest organ site task force is the Breast Cancer Task Force. Traditionally it was administered intramurally upon advice from an Extramural Committee, who were organized to promote multidisciplinary projects in the area of breast cancer. The program covers a spectrum of activities in five sub-areas: diagnosis, epidemiology, experimental biology, therapeutics and information. Spearheading the conversion of contract base basic research program to investigator initiated research, BCTF increased the Grant Program from practically no projects to 46, while decreasing the contract support from 6.3 to 3.1 million in one year.

In addition to these programs, the DCBD's portion of the Frederick Cancer Research Center is managed by the Associate Director for the Extramural Program. This includes the research portion: Cancer Biology Program (Dr. M. Kripke, Director), and Resources Management: Animal Holding Facility (Mr. J. Hatgi, Manager). Reports on FCRC are issued annually by the contractor.

TUMOR BIOLOGY PROGRAM

Description

The Tumor Biology Program was formed in 1974 and is one of the newest and largest program areas within the National Cancer Institute (NCI). Ratification of the National Cancer Act by Congress in 1971 increased the availability of funds for cancer research and resulted in the expansion and diversification of existing grant programs within the Division of Cancer Research Resources and Centers (DCRRC). Because of a need to reduce workloads and management responsibilities of existing programs within DCRRC, a new program, Tumor Biology, was created from parts of the Drug Development Program, Carcinogenesis Program, and Cancer Biology Program. The remainder of the Cancer Biology Program was renamed Viral Oncology. Tumor Biology was to support research that explained differences between normal cells and the end product tumor cells; it would not be concerned with processes that initiated or promoted tumor formation. Since 1974, the biological systems used in research supported by the Tumor Biology Program have changed dramatically. These changes have occurred primarily because we have learned to culture and clone cancer cells effectively; because we have discovered that molecular mechanisms controlling genetic expression in eukaryotes are considerably different from those that occur in bacteria; and because model systems for studying neoplastic processes in living animals have been developed. Thus, bacterial research has completely disappeared from the program while tissue culture studies and animal studies of neoplastic processes have increased at a rapid rate. Although the content of the Tumor Biology Program did not change as a result of the reorganization of the NCI and its incorporation into the Division of Cancer Biology and Diagnosis in 1979, the program has been divided into two Sections with distinct responsibilities for *in vitro* and *in vivo* research, the Basic Cancer Biology Section and the Tumor Biology Section.

The Tumor Biology Program supports a broad spectrum of basic biological and biochemical research (See Appendix I) in the pursuit of one overriding goal, defining properties of cancer cells and tumors that uniquely distinguish them from normal, healthy cells and tissues. The supposition is that if we can define these properties and learn how to manipulate or change the biological signals and/or modify biochemical reactions responsible for the aberrant behavior of cancer cells, applied methods can be developed for the successful treatment, diagnosis and therapy of cancer patients. Within this goal are three major areas of investigation which correspond to different theories of how to control the development and progression of neoplastic disease. The first is understanding the basic biochemical mechanisms involved in growth control, whether these involve particular external signals that initiate the process of cell division or particular internal molecules more directly responsible for DNA replication and metabolism. This kind of information can lead to the development of specific hormonal and drug therapies. The second is studying changes that occur at the molecular level which lead to cancer cell invasion. The invasive behavior of cancer cells is a prerequisite to malignancy, or the

ability of tumors to invade surrounding tissues, escape normal host defense mechanisms and become established at multiple secondary metastatic sites. Theoretically, if the invasive properties of malignant tumors can be controlled and these tumors confined to particular sites, metastasis, the major killer in cancer patients, will not occur. The third is to develop detailed biological and biochemical information about the processes which induce cancer cell differentiation. There is good reason to believe that many kinds of cancers will respond to external stimuli and differentiate. If the genetic program of an actively growing cancer could be changed to one of terminal differentiation, then the malignant tumor could be rendered harmless. Although the above emphasis of the Tumor Biology Program in the areas of growth, invasion and differentiation is stated in simple terms, it provides a convenient and purposeful way of viewing the role of basic biological research to the ultimate goal of curing cancer.

The kinds of information developed in the Tumor Biology Program provide a foundation for and relate directly or indirectly to nearly every other program area within the National Cancer Institute. The importance of basic tumor biology research to the National Cancer Plan is reflected by the large $46 million commitment of the NCI to this program area in FY 1980 (See Table I). Appendix II provides a complete listing of all grants supported by the Tumor Biology Program.

Accomplishments

A pervasive problem for tumor biologists is the extreme morphological and behavioral heterogeneity which appears not only between neoplasms of similar origins but also between cell populations within the same neoplasm. Thus, research within the Tumor Biology Program employs a wide variety of cell and tumor systems in an effort to define properties common to all malignancies. Although this goal has not been realized, considerable progress has been made in the past five years in developing homogeneous cancer cell systems that are amenable to a variety of biological and biochemical studies. Established cell lines and cloned cell strains from various tumors have allowed researchers to focus on specific questions that previously were not technically feasible to answer, ranging from studies of enzymes and regulatory factors to mechanisms of organ-site selectivity during metastasis.

It has been known for some time that most cells are coated with an extracellular matrix of proteins which appears to be of great importance in the formation and maintenance of correct tissue morphology, as well as the regulation of cell-cell interactions and cell movement. Collagen, elastin and glycosaminoglycans have long been recognized as the main components of the extracellular matrix; but, more recently, several new very large proteins have been discovered. Their appearance and disappearance with viral transformation and possible correlation with malignancy has stimulated a great deal of research into understanding their structure and function (Ruoslahti et al., 1980). Fibronectin is the protein which has received the greatest amount of attention because of its high abundance in the

extracellular matrix, as well as in the bloodstream. There is every indication from biochemical studies that fibronectin is a molecule which interacts with glycosaminoglycans (Perkins et al., 1979), is highly glycosylated (Carter and Hakamori, 1979; Fukuda and Hakamori, 1979) and has a special binding site for collagen (Ruoslahti et al., 1979; Wagner and Hynes, 1979). Although these chemical studies are tedious, they will tell us more about how fibronectin regulates the adhesive properties of cells that play a significant role in the morphogenetic events of normal development. More importantly, they will tell us about the mechanisms that anchor cells to the extracellular matrix, which are of obvious importance to the malignant cell's capacity to grow invasively, free from the restraints of attachments to surrounding cells and tissues. However, the overall picture of the extracellular matrix's importance to invasiveness is not limited to fibronectin. Few normal epithelial adult tissues, where cancers are most likely to develop, contain fibronectin. But during the invasive process, malignant cells must penetrate extracellular matrices which often contain fibronectin. In addition, several new proteins have been detected as part of the extracellular matrix and cell membrane which, when altered, may contribute to malignancy (Chen et al., 1979; Kayama et al., 1979). This is an exciting area of investigation which offers considerable promise for the future understanding of how cell-cell and cell-matrix interactions contribute to the invasive process.

Malignant cells possess the abilities to invade surrounding normal tissues, but little is known about the actual mechanism of invasion. Regardless of progress in molecular biology, such as with fibronectin and other proteins of the extracellular matrix, major limitations in understanding the invasive process will remain until good techniques are developed for measuring invasiveness and more biological model systems become available that can be used for both in vitro and in vivo experimentation. Even though there are always problems of interpreting the relevance of in vitro studies to actual in vivo processes, measuring contact inhibition of growth, migratory behavior and differential adhesiveness of cells in vitro are useful determinants of malignancy (Abercrombie, 1979). However, several new systems for measuring invasion have been reported in the last year and hold much promise for cancer research. A critical step in metastasis relies on the ability of malignant cells to attach to and invade vascular endothelial cell layers before reaching the blood stream. Kramer and Nicolson (1979) have developed a model to evaluate malignant tumor cell interactions with vascular endothelial cells. Using B16 melanoma variant cell populations which differ in their metastatic potential and organ-site selectivity, these researchers were able to demonstrate that their in vitro model system mimicked much of what is known about invasion in vivo. They found that the invasive melanoma cells never penetrated directly into endothelial cytoplasms but worked their way between and under the endothelial cells and reestablished intercellular junctions with each other, effectively walling themselves off from the medium in the plastic culture dish. The process could be quantitated in terms of the time required for malignant cells to attach to endothelial cells and the time from attachment to submersion. Poste et al., (1980) have perfected

techniques that can quantitate tumor cell invasion into tissues of different histological complexity maintained as organ cultures. They can measure invasion through the chorioallantoic membrane of chicken, mouse urinary bladder, and canine blood vessels. These conditions provide an excellent way to mimic the in vivo situation, because invasive cells are required to penetrate an organized tissue matrix. Using the same B16 melanoma variants of Nicolson above, they were able to demonstrate that cells which invaded more complex tissues could easily invade the simple structure of the chorioallantoic membrane but not vice versa. In addition, their results suggested that factors responsible for the invasive phenotype in their test system selected for cellular properties required for the successful completion of other steps in the metastatic process. The results of Nicolson and Poste are extremely encouraging and should provide key tools for studying molecular factors and interactions important to the regulation and control of malignancy.

Other research investigating the invasive process at the level of the cell membrane and cell-cell interactions have yielded results which indicate why the invasive process has been so difficult to understand at the molecular level. Again the metastatic variants of the B16 melanoma model have been used to good advantage. A clever experiment was performed which fused membrane vesicles from highly metastatic cells with low frequency metastatic cells and demonstrated that high metastatic potential was transferred to the low metastatic cell population (Poste and Nicolson, 1980). Although these experiments clearly confirm that differences in cell surface properties distinguish high metastatic from low metastatic cell populations, careful comparative biochemical analyses of the membranes show some major qualitative but not quantitative differences (Raz et al., 1980). The same result was found in metastatic variant RNA-virus transformed mouse cell lines and in normal and malignant mammary cells (Yogeeswaran et al., 1979; Chandrasekan and Davidson, 1979). A clear quantitative difference would be much easier to analyze, but these kinds of results may indicate why tumor specific antigens have never been found and why immunotherapy by itself has not proved to be successful. However, other experiments in vitro indicate that B16 melanoma variants of high metastatic potential inhibit aggregation and adhesion of normal cells by releasing a product into the medium (Maslow et al., 1980). Whether this result is a membrane product shed from the cell surface membrane or an enzyme or some other factor secreted from the cells remains to be determined. In any case, there could easily be several factors acting synergistically or in concert during the invasive process.

The communication between cells must be an essential process during development and in the maintenance of normal adult control process. One way in which cells are believed to communicate is directly by diffusion of molecules through specialized cell membrane channels that provide cytoplasmic links between touching cells in vitro and in vivo. A recent, interesting observation may have considerable impact on theories of cell-cell communication. It shows that membrane junctions formed between two different cell types may not result in symmetrical communication between the cells (Flagg-Newton and Loewenstein,

1980). For sometime it was believed that cancer cells failed to form these membrane junctions and that failure to communicate with surrounding cells was one basic reason for the loss of growth control and invasive behavior of malignant neoplasias. However, cancers do form junctional contacts with other cells. The question now arises whether one-way communication between cancer cells and surrounding cells contributes to the cancer cell's autonomous behavior under conditions which are normally restrictive.

There continues to be considerable controversy over whether changes in the cytoskeletal and contractile elements of cancer cells are a result of or possibly a controlling factor in expression of the transformed phenotype in culture (Goldman et al., 1979; Brinkley et al., 1980). Microfilaments and microtubules connect directly or indirectly to macromolecules in the cell surface membrane and participate in regulating a number of membrane-associated events, including cell morphology, cell motility, contact inhibition, cell spreading and adhesive properties, anchorage-dependent growth, mobility of cell surface receptors, and binding of growth factors to cell surface receptors. Because neoplastically transformed cells show alterations in many of these same features, it has been hypothesized that their lack of response to external growth stimuli may be due to changes or modifications in the structure and organization of cytoskeletal and contractile elements. Until recently, however, no one had examined cells freshly derived from adult tissues. Brinkley and his co-workers may have come up with a very significant result. They found that comparison of normal, preneoplastic and neoplastic cells from fresh tissues showed no significant differences in either the number or organization of fibrillar cytoplasmic components. (Asch et al., 1979). Although it still remains important to know how contractile elements work as part of understanding the invasive process, alterations in cytoskeletal elements may no longer be worth studying as a primary event leading to transformation.

In the last few years a startling discovery was made concerning the processing and regulation of messages in eukaryotic cells that code for proteins which are responsible for how cells look and behave. It was discovered that these messages initially contained spacer sequences that were not relevant to the primary sequence of proteins and had to be removed before the message could be used. A whole new level of biochemical control was placed on macromolecular synthetic processes in cells. Now, Dr. Gunter Blobel and his colleagues have discovered that the signal for translocation of secretory peptides across membranes can reside in the middle of the protein molecule rather than at the ends of the molecule (Vishwanath et al., 1979). Important proteins such as fibronectin would have to be secreted into the extracellular matrix. Again, progress in basic molecular biology has changed our whole view of how secretory proteins are translocated through membranes and deposited at their physiologically important sites.

One of the leading proponents of what has been called "the differentiation hypothesis" of cancer is Pierce (1978). He argues that the defect in the malignant cell is that it continues to proliferate rather than proceed through its normal program of maturation, which usually precludes any further cell division. If the proper signals for differentiation can be restored the malignancy should be reversible at the cellular level.

Several laboratories are analyzing tumor cell systems that can be persuaded to display certain "normal" characteristics under specific *in vitro* conditions. It was first reported in 1978 that embryonal carcinoma cells of the F9 strain could be induced to synthesize proteins characteristic of endodermal cells by retinoic acid. More recently Solter *et al.*, (1979) showed that the retinoic acid treated cells also develop antigenic determinants of early mouse embryos and that these changes are irreversible. A human promyelocytic leukemic cell line can be induced to differentiate into macrophage-like cells with either dimethyl sulfoxide (DMSO) or phorbol esters. Specific markers such as acid phosphatase isozymes increase markedly (Verbrodt, et al., 1979). Calabresi, Dexter and Heppner (1979) have demonstrated the effect of N,N-dimethylformamide (DMF) on two different cell lines. Rhabdomyosarcoma cells, when exposed to DMF, begin to express creatine phosphokinase, an enzyme otherwise found only in muscle and brain. Human colon carcinoma cells develop changes in their antigenic determinants more typical of normal cells in the presence of the inducer.

Housman and co-workers (Levenson *et al.*, 1979; Tsifisoglou *et al.*, 1979) (1979) are studying the mechanism of commitment to differentiation by DMSO of mouse erythroleukemia cells, as indicated by new synthesis of heme protein. The DMSO effect can be blocked by dexamethasone, inhibitors of protein synthesis, or cordycepin. Further, the presence of both DMSO and cordycepin promotes synchronization of the culture such that after withdrawal of the inhibitor differentiation follows rapidly in the majority of cells.

Several years ago, Rosenberg and colleagues demonstrated that dibutyryl cAMP treatment of mouse neuroblastoma cells provoked the formation of neurites. Subsequently, similar results were observed in these malignant cells with prostoglandin E_1 and phosphodiesterase inhibitors. The induction of other typical characteristics of neuronal cells, including a many-fold increase in cAMP binding protein, has recently been reported (Prashad *et al.*, 1980). Glucocorticoid treatment of mouse neuroblastoma cells also alters both their biochemistry and their tumorigenicity towards a more differentiated phenotype (Sandquist *et al.*, 1979). Information derived from these rather artificial biological systems may prove to be valuable models on which to base therapeutic studies in live animals. Theoretically, these compounds could help control neoplastic progression *in vivo*.

The tumor biology program supports a substantial program of research on an interesting neoplastic disease called crown gall tumor. This cancer of dicotyledonous plants is caused by the bacterium *Agrobacterium tumefaciens* at

sites of tissue damage. The transforming factor in the bacterium was recently identified as a large plasmid (Ti, tumor inducing). Loss of this plasmid results in loss of the bacterium's tumorigenetic ability. When the plasmid is reintroduced, virulence returns. Transfer of only about 10% of the total plasmid is actually required for neoplastic transformation (Chilton et al., 1977). This active DNA region may contain coded information for the synthesis of as many as ten vital proteins. One or several of these proteins are unique enzymes necessary for the catabolism of octapine or nopaline, energy sources for the cell. These opines (arginine derivatives) are not found in normal plant tissue.

Another interesting property of crown gall tumor tissue is its phenotypic reversion to a normal morphology when the teratoma tissue is grafted onto a normal plant and it then bears seeds that give rise to normal plants (Braun & Wood, 1976). When explanted back into tissue culture the neoplastic phenotype returns (Turgeon et al., 1976).

Crown gall tumor has recently been called a natural case of genetic engineering (Nester and Montoya, 1979) and the mechanism of inducing the neoplasm is very similar to what we know about viral transformation of mammalian cells. It appears to be a system perfectly designed for the current technology in molecular biology and genetics. The sequence of the tumor-inducing DNA, and adjacent DNA sequences in the plasmid, their specific locations within the plant cell and possible mechanisms of stabilization or integration are questions under investigation (Thomashow et al., 1980., Loper and Kado, 1979). Furthermore, several other investigators have recently begun independent projects on crown gall tumor with funds from this program (A. Matthysee, CA 18604; S.K. Farrand, CA 19402; H.M. Goodman, CA 27424).

REFERENCES

Abercrombie, M., "Contact Inhibition and Malignancy," Nature, 281: 259-262, 1979. (No Grant Number)

Asch, B.B., Medina, D., and Brinkley, B.R., "Microtubules and Actin-containing Filaments of Normal, Preneoplastic and Neoplastic Mouse Mammary Epithelial Cells," Cancer Research, 39: 893-907, 1979. (CA 22610)

Braun, A.C., and Wood, H.N., "Suppression of the Neoplastic State with the Acquisition of Specialized Functions in Cells, Tissues and Organs of Crown Gall Teratomas of Tobacco," Proc. Natl. Acad. Sci., U.S.A., 73:496-500, 1976. (CA 13808)

Brinkley, B.R., Fistel, S.H., Marcum, J.M., and Pardue, R.L., "Microtubules in Cultured Cells; Indirect Immunofluorescent Staining with Tubulin Antibody," International Rev. of Cytology, 63: 59-92, 1980. (CA 22610)

Calabresi, P., Dexter, D.L., and Heppner, G.H., "Clinical and Pharmacological Implications of Cancer Cell Differentiation and Heterogeneity," Biochem. Pharm. 28:1933-1941, 1979. (CA 27419)

Carter, W.G., Hakomori, S., "Isolation of Galactoprotein a from Hamster Embryo Fibroblast and Characterization of the Carbohydrate Unit," Biochemistry, 18, 730-738, 1979. (CA 23907)

Chilton, M.-D., Drummond, M.H., Merlo, D.J., Schiaky, D., Montoya, A.L., Gordon, M.P., and Nester, E.W., "Stable Incorporation of Plasmid DNA into Higher Plant Cells: The Molecular Basis of Crown Gall Tumorigenesis," Cell, 11:263-271, 1977. (CA 13015)

Chung, A.E., Jaffe, R. Freeman, I.L., Vergnes, J-P., Braginski, J.E., and Carlin, B., "Properties of a Basement Membrane-Related Glycoprotein Synthesized in Culture by a Mouse Embryonal Carcinoma-Derived Cell Line," Cell, 16: 277-287, 1979. (CA 21246)

Flagg-Newton, J.L., and Loewenstein, W.R., "Asymmetrically Permeable Membrane Channels in Cell Junction," Science, 207: 771-773, 1980. (CA 14464)

Fikuda, M., and Hakomori, S., "Carbohydrate Structure of Galactroprotein a, a Major Transformation Sensitive Glycoprotein Released from Hamster Embryo Fibroblasts," J. Biol. Chem., 254: 5451-5457, 1979. (CA 23907)

Goldman, R.D., Milsted, A., Schloss, J.A., Starger, J., and Yerna, M-J., "Cytoplasmic Fibers in Mammalian Cells: Cytoskeletal and Contractile Elements," Ann. Rev. Physiol., 41: 703-722, 1979. (CA 17210)

Koyama, K., Nudelman,E., Fukuda, M., and Hakomori, S., "Correlation of Glycosylation in a Membrane Protein with a Molecular Weight of 150,000 with Tumorigenic Property of Rat Fibrosarcoma Variants," Cancer Research, 39: 3677-3682, 1979. (CA 14464)

Kramer, R.H., and Nicolson, G.L., "Interaction of Tumor Cells with Vascular Endothelial Cell Monolayers: A Model for Metastatic Invasion," Proc. Natl. Acad. Sci., U.S.A., 76: 5704-5708, 1979. (CA 15122)

Levenson, R., and Housman, D., "Developmental Program of Mouse Erythroleukemia Cells," J. Cell Biol. 82:715-725, 1979. (CA 17575)

Levenson, R., Kernen, J., and Housman, D., "Synchronization of MEL Cell Commitment with Cordycepin," Cell 18:1073-1078, 1979. (CA 17575)

Loper, J.E., and Kado, C.I., "Host Range Conferred by the Virulence Specifying Plasmid of Agrobacterium Tumefaciens," J. Bacteriol. 139: 591-596, 1979. (CA 11526)

Maslow, D.F., Mayhew, E., and Feldman, J., "Differential Inhibition of In Vitro Malignant Cell Assays by B16 Mouse Melanoma Variants," J. Natl. Cancer Inst, 64: 635-637, 1980. (CA 17609)

Nester, E.W., and Montoya, A., "Crown Gall: A Natural Case of Genetic Engineering," ASM News 45:283-287, 1979. (CA 13015)

Perkins, M.E., Ji, T.H. and Hynes, R.O., "Cross-Linking of Fibronection to Sulfated Proteoglycans at the Cell Surface," Cell, 16: 941-952, 1979. (CA 17007)

Pierce, G.B., Shikes, R.H., and Fink, L.M., CANCER: A Problem of Developmental Biology, Prentice-Hall, Inc., Englewood Cliffs, N.J., 1978. (CA 24010) (CA 15823)

Poste, G., Doll, J., Hart, I.R., and Fidler, I.J., "In Vitro Selection of Murine Melanoma Variants with Enhanced Tissue-Invasive Properties," Cancer Research, 40: 1636-1644, 1980. (CA 18260)

Poste, G., and Nicolson, G.L., "Arrest and Metastasis of Blood-Borne Tumor Cells are Modified by Fusion of Plasma Membrane Vesicles from Highly Metastatic Cells," Proc. Natl. Acad. Sci., U.S.A., 77:399-403, 1980. (CA 15122)

Prashad, N., Evetts, C., Wischmeyer B., and Rosenberg, R.N., "Phosphorylation of Endogenous Proteins by Adenosine 3':5' -Monophosphate-dependent Protein Kinase in Mouse Neuroblastoma Cells," J. Neurochem, July, 1980. (CA 21602)

Prashad, N., Rosenberg, R., Baskin, F., Sparkman, D., Ulrich, C. and Wischmeyer, B., "Cyclic Adenosine 3':5'-Monophosphate-binding Protein, a Biochemical Marker of Neuroblastoma Differentiation," Cancer Research, July, 1980. (CA 21602)

Raz, A., McLellan, W.L., Hart, I.R., Bucana, C.D., Hoyer, L.C. Sela, B.-A., Dragsten, P., and Fidler, I.J., "Cell Surface Properties of B16 Melanoma Variants with Differing Metastatic Potential," Cancer Research, 40:1645-1651, 1980. (No Grant Number)

Ruoslahti, E., Hayman, E.G., and Engvall, E., "Fibronectin," from Cancer Markers, ed. by Stewart Sell, The Humana Press, pp. 85-505, 1980. (CA 27455)

Ruoslahti, E., Hayman, E.G., Kuusela, Shively, J.E., and Engvall, E., "Isolation of a Tryptic Fragment Containing the Collagen Binding Site of Plasma Fibronectin," J. Biol. Chem., 254: 6054-6059, 1979. (CA 27455)

Sandquist, D., Black, A.C., Sahu, S.K., Williams, L. and Williams, T.H., "Dexamethasane Induces Increased Catecholamine Biosynthesis in Cultured Neuroblastoma," Exper. Cell Res., 123:417-421, 1979. (CA 24241)

Solter, D., Shevinsky, L., Knowles, B.B., and Strickland, S., "The Induction of Antigenic Changes in a Teratocarcinoma Stem Cell Line (F9) by Retinoic Acid," Develop. Biol. 70:515-521, 1979. (CA 17546)

Thomashow, M.F., Wood, H.N. and, Braun, A.C., "Studies on the Recovery of Crown Gall Tumor Cells," Proc. Natl. Acad. Sci., U.S.A. 73:3562-3564, 1976.

Tsiftsoglou, A.S., Gusella, J.F., Volloch, V. and Housman, D.E., "Inhibition by Dexamethasone of Commitment to Erythroid Differentiation in Murine Erythroleukemia Cells." Cancer Res. 39:3849-3855, 1979. (CA 17575)

Turgeon, R., Wood, H.N. and Braun, A.C., "Studies on the Recovery of Crown Gall Tumor Cells," Proc. Natl. Acad. Sci., U.S.A. 73:3562-3564, 1976. (CA 23261)

Vishwanath, R.L., Lingapappa, J.R., and Blobel, G., "Chicken Ovalbumin Contains an Internal Signal Sequence, Nature, 281:117-120, 1979.

Vorbrodt, A., Meo, P., and Rovera, G., "Regulation of Acid Phosphatase Activity in Human Promyelocytic Leukemia Cells Induced to Differentiate in Culture," J. Cell Biol., 83:300-307, 1979. (CA 21069)

Wagner, D.D., and Hynes, R.O., "Domain Structure of Fibronectin and Its Relation to Function," J. Biol. Chem., 254:6746-6754, 1979. (CA 17007)

APPENDIX I

TUMOR BIOLOGY REFERRAL GUIDELINES

The Tumor Biology Program supports a broad spectrum of research with animal and human cell systems which includes fundamental and comparative studies in histology, pathology, cell biology, developmental biology, molecular biology, embryology, biochemistry, and genetics. The goal of this program is to develop a better understanding of the biological processes in tumors and tumor cells that are responsible for uncontrolled growth and invasion of surrounding tissues. The majority of studies are aimed at determining characteristic differences in structure, function, biochemical composition and molecular mechanisms between normal and neoplastic cells or between progressive states of tumor development and differentiation. Areas supported include:

- Tumor Progression (e.g., angiogenesis, neovascularization, invasiveness, metastasis)
- Differentiation and Neoplasia (e.g., teratomas, teratocarcinomas, hepatomas, neuroblastomas, melanomas, Friend leukemias)
- Genetics of Neoplasia (e.g., karyotypes, somatic cell genetics, somatic cell hybrids, gene mapping, inherited genetic syndromes)
- Normal, Abnormal and Neoplastic States of Growth (e.g., cell growth cycle, fast growing vs. slow growing)
- Normal and Neoplastic Cell Behavior (e.g., cell-cell adhesions, cell movement and migration-microtubules-microfilaments, cell-cell communication)
- Membrane Synthesis, Structure, Composition and Function (e.g., transport, receptors, sugar transferases, glycolipids, glycoproteins, basement membrane, gap junctions, tight junctions)
- Cell Metabolism - Regulation of (e.g., energy metabolism, metabolic pathways, enzymes, cGMP, cAMP)
- Nucleic Acid and Protein Synthesis and Processing, Interactions and Modifications - Regulation of (e.g., DNA, rRNA, sRNA, mRNA, regulation of gene expression, histones, nonhistones, chromatin)
- Nutritional, Hormonal and Protein Factor Requirements for Tumor Growth, Maintenance and Differentiation (e.g., serum factors, peptide hormones, steroid hormones)
- Model Systems for Studying Tumor Growth, Invasiveness and Metastasis

EXCEPTIONS AND QUALIFICATIONS:

Excluded from this program are all research studies focused on breast cancer as well as those oriented toward detection and diagnosis and immunology.

Experimental studies with prokaryotic and plant systems are supported only when these studies are clearly defended in terms of potential knowledge that will enhance cancer research. Except for comparative studies of normal and virally transformed cells in culture, this program is not concerned with the occurrence, nature or mechanism of action of virally induced cancers.

FISCAL YEAR 1980

TUMOR BIOLOGY PROGRAM

SUMMARY BY SUB-CATEGORY (DOLLARS IN THOUSANDS)

	NON-COMPETING		COMPETING		TOTAL	
	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
Cell Surface	56	$ 5,206	20	$ 1,688	76	$ 6,894
Enzymes	43	2,687	11	890	54	3,577
Peptide Hormones	15	1,308	1	92	16	1,400
Steroids	11	870	6	659	17	1,529
Membraneous Organelles	9	1,452	2	488	11	1,940
Ribosomes and Polyribosomes	6	752	2	269	8	1,021
M-RNA	11	1,168	5	627	16	1,795
T-RNA	3	288	7	645	10	933
DNA	16	1,450	3	212	19	1,662
Growth Factors	9	775	8	891	17	1,666
Nucleus	13	1,036	7	621	20	1,657
Contractile Elements	5	461	1	75	6	536
Development and Differentiation	36	2,939	17	1,837	53	4,776
Cell Growth, Cell Division	29	2,511	11	798	40	3,309
Somatic Cell Genetics	7	878	4	400	11	1,278
Inheritance of Neoplasms	1	67	0	0	1	67
Plasmids, Viruses	4	392	2	295	6	687
In Vivo and In Vitro Tumor Lines	7	595	1	65	8	660
Difficult-to-Classify	7	786	9	632	16	1,418
Sub-Total	288	25,621	117	11,184	405	36,805
Program Projects	10	6,229	6	2,300	16	8,529
Conferences	0	0	4	195	4	195
Young Investigators	12	395	5	176	17	571
Sub-Total	22	6,624	15	2,671	37	9,295
Total	310	$ 32,245	132	$13,855	442	$46,100

R01-CA-02758 Steroid Metabolism in Tumors and Normal Tissues
Andrew A. Kandutsch Jackson Laboratory

R01-CA-08759 The Structure and Biologic Function of Glycoproteins
Stuart A. Kornfeld Washington University

R01-CA-10625 Membrane Permeability of the Ascites Tumor Cells
Felice B. Aull New York University

R01-CA-10917 Phosphate Transport and Metabolism in Carcinoma Cells
Charles Levinson University of Texas Health Science Center

R01-CA-12150 Unusual Lipids in Cancer Tissues
Wolfgang J. Baumann University of Minnesota

R01-CA-12306 Preparation of Cancer Chemotherapy Abstracts, Volume XI
Dennis D. Cunningham University of California, Irvine

R01-CA-12753 Cell Surfaces and Cells Transformed by Oncogenic Viruses
William J. Grimes University of Arizona

R01-CA-12790 Substrate and Cation Transport in Human Leukocytes
Marshall A. Lichtman University of Rochester

R01-CA-12920 Molecular Basis of Intercellular Adhesion
Stephen B. Oppenheimer California State University

R01-CA-13402 Surface Membranes in Normal and Cancer Cells
Paul H. Atkinson Yeshiva University

R01-CA-13605 Chemistry and Measurement of Intercellular Adhesion
Malcolm S. Steinberg Princeton University

R01-CA-14370 Specificity of Normal and Cancer Cell Interactions
David E. Maslow Roswell Park Memorial Institute

R01-CA-14431 Cell Adhesion of Normal and Malignant Liver Cells
Edward J. McGuire National Jewish Hospital & Research Center

R01-CA-14464 A Program on the Etiology and Diagnosis of Cancer
Werner R. Loewenstein University of Miami

R01-CA-14496 Metal Ion Function in Concanavalin A
James A. Magnuson Washington State University

R01-CA-14551 Penetration of Macromolecules into Mammalian Cells
Huques J-P. Ryser Boston University

R01-CA-14609 Adhesion of Normal and Malignant Mammalian Cells
Frederick L. Grinnell University of Texas Health Science Center

R01-CA-14764 Glycolipid Metabolism in Tumor and Transformed Cells
Sabash C. Basu University of Notre Dame

R01-CA-15047 O-Alkyl Lipids in Surface Membranes of Tumor Cells
Samuel J. Friedberg University of Texas Health Science Center

R01-CA-15122 Cell Surface Studies of Metastatic Melanoma
Garth L. Nicolson University of California, Irvine

R01-CA-15483 Biochemistry of Mucopolysaccharides
Eugene A. Davidson Pennsylvania State University

R01-CA-16335 Cell Junction Formation & Role in Cell Proliferation
Judson D. Sheridan University of Minnesota

R01-CA-16865 Analysis of Eucaryotic Cell Surfaces
Carl G. Hellerqvist Vanderbilt University

R01-CA-17007 Cell Surface Structure and Transformation
Richard O. Hynes Massachusetts Institute of Technology

R01-CA-17015 Glycosylation Mechanisms of Cell Lipids and Proteins
Carlos B. Hirschberg St. Louis University

R01-CA-17149 Proteins of the Hepatoma Cell Plasma Membrane
Darrell J. Doyle New York State Department of Health

R01-CA-18260 Modification of Cell Behavior by Lipid Vesicles
George H. Poste Roswell Park Memorial Institute

R01-CA-18801 Glycosphingolipid Metabolism and Tumorigenesis
D. James Morre Purdue University

R01-CA-19015 Sugars, Glycoproteins: Normal and Malignant Cells
Harold Amos Harvard University

R01-CA-19017 Cell Adhesion Proteins and Malignancy
Robert J. Klebe University of Texas Medical Branch

R01-CA-19127 Liposomes as Carriers for Antitumor Agents
Vilma K. Jansons College of Medicine and Dentistry of New Jersey

R01-CA-19130 The Surface Membranes of Normal and Cancer Cells
Leonard Warren Wistar Institute of Anatomy and Biology

R01-CA-19144 Membrane Changes Caused by Tumor Virus Transformation
Clayton A. Buck Wistar Institute of Anatomy and Biology

R01-CA-19158 Mammalian Collagenase in Tumor Invasion
Chitra Biswas Massachusetts General Hospital

R01-CA-19757 Glycoprotein Shedding and Metastasis
H. Bruce Bosmann University of Rochester

R01-CA-20026 Immunospecific Glycolipids of Normal and Cancer Cells
Sen-Itiroh Hakomori Fred Hutchinson Cancer Research Center

R01-CA-20323 Control of Cellular Mobility
Howard Gershman Case Western Reserve University

R01-CA-20421 Mutants Altered in Glycosylation of Membrane Proteins
Sharon S. Krag Johns Hopkins University

R01-CA-20424 Murine Ascites Tumor Cell Glycoproteins
Irwin J. Goldstein University of Michigan

R01-CA-20575 Myeloid Cell Surface Receptors: Normal & Leukemic
Sondra G. Barrett University of California, San Francisco

R01-CA-21463 Pathobiology of the Cell Membrane in Cancer
Leo T. Furcht University of Minnesota

R01-CA-21923 Oligosaccharide Structure and Receptor Specificity
Jacques Baenziger Washington University

R01-CA-22451 Contact Behavior of Developing and Transformed Cells
John P. Trinkaus Yale University

R01-CA-22659 Studies of Proteins Involved in Cell Interaction
Lan B. Chen Sidney Farber Cancer Institute

R01-CA-22729 Hormonal Regulation of Membrane Phenotype
Thomas D. Gelehrter University of Michigan

R01-CA-22857 Mammalian Glycoprotein Oligosaccharides
Charlene G. Gottlieb Washington University

R01-CA-22907 Collagenase and Invasion of Head and Neck Tumors
Cheng-Chun Huang Columbia University

R01-CA-23016 Animal Cell Surface Glycosaminoglycans
John N. Keller University of Health Science/Chicago Medical School

R01-CA-23095 Carcinogenesis/Membrane Enzyme & Permeability Mutants
Joy Hochstadt New York Medical College

R01-CA-23540 Collagen and Its Relationship to Tumors
Barbara D. Smith Boston University

R01-CA-23753 Membrane Proteins of Normal and Malignant Cells
Daniel B. Rifkin New York University

R23-CA-23838 Chemistry of Drug Delivery by Lipid Vesicles
Leslie M. Loew State University of New York

R01-CA-23907 Galactoprotein A and B in Normal and Transformed Cells
Sen-Itiroh Hakomori Fred Hutchinson Cancer Research Center

R01-CA-24051 Synthetic Glycosides for Cancer Research
Khushi L. Matta Roswell Park Memorial Institute

R01-CA-24090 Electrophoretic Separation of Gamma-Irradiated Cells
Paul W. Todd Pennsylvania State University

R23-CA-24241 Differentiation in a Malignant Neural Tumor
Dean S. Sandquist University of Iowa

R01-CA-24339 Membrane Lipid Asymmetry in a Tumorigenic Cell Line
Friedhelm Schroeder University of Missouri

R01-CA-24419 Role of Glycoconjugates in Formation of Metastasis
Vladimir P. Skipski Sloan Kettering Institute for Cancer Research

R01-CA-24439 Macromolecules for Isolating Protein on Cell Surfaces
Jules A. Shafer University of Michigan

R01-CA-24553 Targeting of Liposomes to Tumor Cells
Leaf Huang University of Tennessee

R01-CA-24598 Mechanism of Radiogallium Uptake by EMT-6 Sarcoma
Stephen M. Larson University of Washington

R01-CA-24605 Fibronectin and Its Loss in Malignant Transformation
Antti I. Vaheri University of Helsinki

R01-CA-25074 Cell Surface Alterations in Malignancy
Milton M. Weiser State University of New York

R01-CA-25532 Glycolipids of Normal & Transformed Mouse Lymphocytes
Gerald A. Schwarting Eunice Kennedy Shriver Center Mental Retardation

R01-CA-25626 Biochemical Probes of Malignant Cell Surfaces
Philip G. Phillips U.S. Veterans Administration

R01-CA-25730 Membrane Proteins and Lipids in Mammary Carcinoma
Marion R. Steiner University of Kentucky

R01-CA-25898 Analysis of G1 in Mammalian Cells
Renato L. Baserga Temple University

R01-CA-26055 Structure-Function Relationships of Antitumor Lectins
Elizabeth F. Fowler University of North Carolina

R01-CA-26085 Ecto 5'-Nucleotidase as a Cell Surface Reporter
Kermit Carraway Oklahoma State University

R01-CA-26103 Plasma Membrane Synthesis in Friend Leukemic Cells
Richard C. Hunt University of Mississippi Medical Center

R01-CA-26122 Effect of Hormones on Cell Surface Architecture
Heinz Baumann New York State Department of Health

R01-CA-26294 Glycoproteins of Normal/Malignant Human Blood Cells
Carl G. Gahmberg University of Helsinki

R01-CA-27285 Plasma Membranes and Control of Cell Growth
Scott W. Peterson Boston University

R01-CA-27389 Cell Surface Fibrous Proteins & Control of Metastasis
Irwin I. Singer Institute for Medical Research

R01-CA-27397 Plasma Membrane Alterations in Transformed Cells
Louis M. Fink University of Colorado Medical Center

R01-CA-27441 Cell Surface Changes in Epidermal Differentiation
Miriam M. Brysk University of Texas Medical Branch

R01-CA-27455 Fibroblast Surface Antigen
Erkki I. Ruoslahti La Jolla Cancer Research Foundation

R01-CA-27460 Alpha-Fetoprotein: Structure and Function
Erkki I. Ruoslahti La Jolla Cancer Research Foundation

R01-CA-27465 Antioxidants in Mouse Neuroblastoma
Richard M. Arneson Oak Ridge Associated Unversities

R01-CA-27467 Study of Basement Membrane Glycoprotein and Collagen
Lewis D. Johnson University of South Carolina

R01-CA-27648 Tumorigenesis and a Cell Surface Growth Inhibitor
Terry C. Johnson Kansas State University

R01-CA-27989 Chronic Myelocytic Leukemia Pathogenesis & Therapy
Robert N. Taub Virginia Commonwealth University

R01-CA-28101 Glycosaminoglycans in Normal and Malignant Cells
Erkki I. Ruoslahti City of Hope National Medical Center

R01-CA-28287 Driving Forces for Nutrient Transport in Tumor Cells
Thomas C. Smith University of Texas Health Science Center

R01-CA-28548 Developing Immunological Probes for Gap Junctions
Ross G. Johnson University of Minnesota

R01-CA-28685 Phosphorylation Events and Transformed Cell Membranes
Julius A. Gordon University of Colorado Medical Center

R23-CA-29522 Diffraction Analysis of Macromolecular Assemblies
Lee Makowski Columbia University

R01-CA-29571 Malignant Cell Variants of Lymphosarcoma
Garth L. Nicolson University of Texas System Cancer Center

ENZYMES

R01-CA-02790 Biosynthesis and Interconversion of Purines
Joseph S. Gots University of Pennsylvania

R01-CA-03728 The Metabolism of Normal and Leukemic Leukocytes
William S. Beck Massachusetts General Hospital

R01-CA-04679 Biology of Normal and Malignant Melanocytes
Aaron B. Lerner Yale University

R01-CA-10916 Metabolism of Normal and Neoplastic Tissues
Sidney Weinhouse Temple University

R01-CA-11655 Studies of Leukocyte Metabolism
Robert Silber New York University

R01-CA-11683 Studies on Coenzymes and Nucleic Acid Metabolism
Nathan O. Kaplan University of California, San Diego

R01-CA-11949 Ether Lipids in Cancer-Enzyme Mechanisms & Inhibitors
Fred Snyder Oak Ridge Associated Universities

R01-CA-12563 Mechanism of Enzyme Induction by Cyclic Nucleotides
Robert H. Stellwagen University of Southern California

R01-CA-14881 Regulation of Tyrosine Synthesis in Hepatoma Cells
Ross Shiman Pennsylvania State University

R01-CA-15196 Regulation of Fatty Acid Biosynthesis in Mammary Tumors
Fazal Ahmad Papanicolaou Cancer Research Institute

R01-CA-15356 Prostaglandin Synthesis in Transformed Cells
J. Martyn Bailey George Washington University

R01-CA-16184 Regulation of Enzyme Levels in Mouse Hepatoma
Michael B. Felder University of South Carolina

R01-CA-16231 The Role of Proteolysis in Malignant Growth
Irvin E. Liener University of Minnesota

R01-CA-16460 Idurd and Alkaline Phosphatase in Human Cancer Cells
Barry Goz University of North Carolina

R01-CA-20600 Porphyrin Specificity in Malignancy
Thomas J. Dougherty Roswell Park Memorial Institute

R01-CA-21967 Fundamental Enzymologic Studies Applied to Oncology
William H. Fishman La Jolla Cancer Research Foundation

R01-CA-22301 Interactions of Vibrio Cholera Enterotoxin
Marc W. Bitensky Yale University

R01-CA-22329 Reduction of Ribonucleotides in Normal and Tumor Tissues
Jay S. Roth University of Connecticut

R01-CA-22409 Enzyme Regulation in Normal Neoplastic Cells
Thomas Deuel Jewish Hospital of St. Louis

R01-CA-22472 Fetal Liver and Hepatoma in Organ Culture
Carl Monder Joint Diseases North General Hospital

R01-CA-22690 Cyclic Nucleotides in Cancer and Regenerating Tissue
Edward N. Rosenberg Albany Medical College

R01-CA-22717 Regulation of Folate Poly-Y Glutamate Synthesis
Barry Shane Johns Hopkins University

R01-CA-23391 Cyclic GMP System in Cell Proliferation
Namoru Shoji Emory University

R01-CA-23743 Ornithine Decarboxylase Induction in Neoplastic Cells
Craig V. Byus University of California, Riverside

R01-CA-24150 Microanalysis of cAMP Enzymes in Human Breast Tumors
Charles L. Rutherford Virginia Polytechnic Institute and State University

R01-CA-24966 Catalase Isozymes in Normal and Neoplastic Tissues
Edwin T. Nishimura U.S. PHS Hospital

R01-CA-25005 The Regulation of Mammalian Enzyme Synthesis
Olga Greengard Mount Sinai School of Medicine

R01-CA-25088 Human Pancreatic Ribonuclease and Cancer
Dohn G. Glitz University of California, Los Angeles

R01-CA-25337 Drugs and Flavoprotein Mediated Superoxide Formation
Garth Powis Mayo Foundation

R01-CA-25473 Cyclic AMP Metabolism in Cultured Epithelial Cells
Richard M. Niles Boston University

R01-CA-25617 The Collagenolytic System of Invasive Tumors
Mustafa K. Dabbous University of Tennessee Center Health Science

R01-CA-26033 White Blood Cell Function in Hematologic Disorders
David G. Nathan Children's Hospital Medical Center

R01-CA-26036 Protein Kinases in Normal and Leukemic Lymphocytes
Orlando J. Martelo University of Cincinnati

R01-CA-26102 Protein Kinase Activity in Rapid Growing Hepatoma Tissue
Wayne Criss Howard University

R01-CA-26470 Regulation of Dihydrofolate Reductase Gene Expression
Lee F. Johnson Ohio State University

R01-CA-26546 Regulation of Enzyme Activity in Normal & Tumor Cells
Evangelo S. Canellakis Yale University

R01-CA-27073 Cellular Enzyme Changes in Childhood Leukemia
Nancy L. Dunn Virginia Commonwealth University

R01-CA-27491 Form and Function of Normal and Neoplastic Ferritins
William H. Massover College of Medicine and Dentistry of New Jersey

R23-CA-27500 The Control of Cyclic GMP in Human Lymphocytes
Dolores J. Takemoto Kansas State University

R01-CA-27572 Ribonucleotide Reductase of Tumor Cells
Joseph G. Cory University of South Florida

R01-CA-27674 Control of Pyrimidine Biosynthesis in Mammalian Cells
David R. Evans Wayne State University

R01-CA-28111 Hormonal Regulation of Kidney Epithelial Cell Growth
Mary L. Taub University of California, San Diego

R01-CA-28376 Nucleotide Biosynthesis and Degradation
Robert Silber New York University

R01-CA-28725 Asparagine Biosynthesis in Normal and Tumor Cells
Sheldon M. Schuster University of Nebraska

R01-CA-28781 Dolichyl Phosphate Biosynthesis in Tumor Cells
W. Lee Adair University of South Florida

R01-CA-28803 The Role of AP4A in Malignant Transformation
Eliezer Rapaport Boston University

R01-CA-28872 Peroxidase in Normal and Neoplastic Melanogenesis
Milton R. Okun Dermatopathology Foundation

PEPTIDE HORMONES

R01-CA-01915 Biologic Characteristics of Thyroid Cancer
Colin G. Thomas University of North Carolina

R01-CA-02146 Cytoplasmic Factors in Cellular Growth
Nancy L. Bucher Massachusetts General Hospital

R01-CA-07535 Influence of Hormones on Tissue Metabolism
Robert N. Macleod University of Virginia

R01-CA-11685 Tumor Cell Synthesis & Secretion of Peptide Hormones
David N. Orth Vanderbilt University

R01-CA-16417 Pituitary Hormones in Normal and Neoplastic Growth
Janakiraman Ramachandran University of California, San Francisco

R01-CA-17309 Prostaglandins & Enzymes in Normal and Malignant Tissues
Lawrence Levine Brandeis University

R01-CA-17389 C-Cell Hyperplasia and Thyroid Medullary Carcinoma
Hubert J. Wolfe Tufts University

R01-CA-18404 Histaminase and Amines in Neural Crest Tumors
Stephen Baylin Johns Hopkins University

R01-CA-19234 Extrinsic Fluorescence and Membrane Potentials
Philip C. Laris University of California, Santa Barbara

R01-CA-22394 Hormonal Control of Cell Proliferation
E. Aubrey Thompson University of South Carolina

R01-CA-23185 Regulation of Alpha and Beta Subunits of Human TSH
Ione A. Kourides Sloan Kettering Institute for Cancer Research

R01-CA-23248 Prolactin Cell Function in Breast Cancer
Wesley C. Hymer Pennsylvania State University

R01-CA-24050 ACTH Secretion by Pituitary Tumor Cells in Culture
U. Ingrid Richardson Harvard University

R01-CA-24604 Triiodothyronine Receptors and Nonthyroidal Diseases
Martin I. Surks Montefiore Hospital and Medical Center

R01-CA-25614 Antiestrogen Action and Cancer
Kenneth I. Williams Worcester Foundation for Experimental Biology

R01-CA-25820 Receptors & Growth Factors for Neoplastic Cells
Joseph Schlessinger Weizmann Institute of Science

R01-CA-28826 Mutations Affecting Gene Expression in Tumor Cells
Robert B. Ivarie University of Georgia

STEROIDS

R01-CA-02071 Estrogen Metabolism and Action in Pregnancy and Cancer
Mortimer Levitz New York University

R01-CA-07057 Steroid Hydroxylases in Normal and Abnormal Adrenals
Boyd W. Harding University of Southern California

R01-CA-08315 Steroid Induced Changes in Cultured Malignant Cells
George Melnykovych University of Kansas

R01-CA-13410 Mechanism of Hormone Action on Target Cells in Culture
Carlos Sonnenschein Tufts University

R01-CA-16091 Biochemical Control in Adrenocortical Carcinoma Cells
Rameshwar K. Sharma University of Tennessee Center Health Science

R01-CA-17323 Glucocorticoid-Resistant Leukemic Lymphocytes
Allan U. Munck Dartmouth College

R01-CA-19907 Physiology of Pituitary Cell Glucocorticoid Binding
Robert W. Harrison Vanderbilt University

R01-CA-22246 Control of Prostate Cell Growth and Cell Division
Kenning N. Anderson Rush-Presbyterian-St. Lukes Medical Center

R01-CA-23603 Gonadotropin Actions in Leydig Tumor Cells
Mario Ascoli Vanderbilt University

R01-CA-23921 Biochemical and Clinical Aspects of Steroid Receptors
Antonio E. Colas University of Wisconsin

R01-CA-24228 Endocrine Factors in Cancer-Induced Lipolysis
Edwin A. Zepp Kirksville College of Osteopathic Medicine

R01-CA-24347 Hormone Effects on Proliferation of Malignant Thymocytes
E. Aubrey Thompson University of South Carolina

R01-CA-25365 Hormonal Regulation of Cultured Endometrial Cells
Lazaro E. Gerschenson University of Colorado Medical Center

R23-CA-25655 Protein Mediators of Lethal Glucocorticoid Effects
Mary L. Nicholson University of Rochester

R01-CA-25974 Model for the Study of Trophoblastic Neoplasia
Larry Boots University of Alabama

R01-CA-26020 Corticosteroids--Cytokinetic and Biochemical Studies
Paul G. Braunschweiger Allegheny-Singer Research Corporation

R01-CA-26112 Effect of Estrogen on Normal and Abnormal Cell Growth
James H. Clark Baylor College of Medicine

R01-CA-26617 Estrogen Mediated Pituitary Tumor Cell Growth
David A. Sirbasku University of Texas Health Science Center

R01-CA-26638 Cyclic AMP: Role in Adrenal Tumor Steroidogenesis
William R. Moyle Rutgers Medical School

R01-CA-27702 Sex Hormones, Cancer and the Immune System
Pentti K. Siiteri University of California, San Francisco

R01-CA-27828 Pituitary Steroid Receptors, Estrogens, and Adenomas
Arnold J. Eisenfeld Yale University

R23-CA-28580 Therapeutic Implications of Hormone in Nevi and Melanoma
Prabir K. Chaudhuri University of Illinois Medical Center

MEMBRANEOUS ORGANELLES

R01-CA-06576 Biological Effects of a Placental Protease Inhibitor
Alex B. Novikoff Yeshiva University

R01-CA-08964 Energy Metabolism in Normal and Tumor Cells
Ephraim Racker Cornell University

R01-CA-10951 Control of Enzymatic Phosphate Transfer in Mitochondria
Peter L. Pedersen Johns Hopkins University

R01-CA-11766 Metabolic Regulation in Neoplasms by Cation Transport
Ronald S. Cockrell St. Louis University

R01-CA-12312 Mitochondrial DNA in Malignant Cells
David A. Clayton Stanford University

R01-CA-12410 The Role of Yeast Mitochondria and Cancer
Julius Marmur Yeshiva University

R01-CA-12858 Lysosome Biogenesis: Normal and Tumor Cells
Philip D. Stahl Washington University

R01-CA-13814 Mitochondrial Genetic System in Normal & Cancer Cells
Margot M. Nass University of Pennsylvania

R01-CA-16527 Regulation of Mitochondrial DNA Replication
Donald L. Robberson University of Texas System Cancer Center

R01-CA-25360 Respiration-Coupled Transport Processes in Tumor Cells
Albert L. Lehninger Johns Hopkins University

R01-CA-28677 Transport in Cholesterol-Rich Tumor Mitochondria
Peter S. Coleman New York University

RIBOSOMES

R01-CA-04186 Molecular Structure of Nucleic Acids and Proteins
Alexander Rich Massachusetts Institute of Technology

R01-CA-08416 Transcription and Translation in Mammalian Cells
Sheldon Penman Massachusetts Institute of Technology

R01-CA-10628 Comparative Structure and Function of Ribosomes
Calvin S. McLaughlin University of California, Irvine

R01-CA-16608 Translation Control in Reticulocytes and Leukemic Cells
Boyd A. Hardesty University of Texas

R01-CA-21663 Intermediary Metabolism in Animals and in Man
Edgar C. Henshaw University of Rochester

R01-CA-23632 RNA Methylation: A Paradox in Normal and Cancer Cells
Thomas O. Sitz Old Dominion University

R01-CA-25618 RDNA-Binding Proteins and Control of RDNA in Tumors
James C. Bearden University of Hawaii

R01-CA-28513 Structure of Hypomethylated Tumor 5.8S Ribosomal RNA
Donald R. Lightfoot Eastern Washington University

M-RNA

R01-CA-12411 Post-Transcriptional Control in Eukaryotic Cells
Thomas E. Webb Ohio State University

R01-CA-12550 RNA Synthesis and Transport in Mammalian Cells
Terence E. Martin University of Chicago

R01-CA-13175 Control of RNA Processing in Normal and Tumor Cells
Fritz M. Rottman Michigan State University

R01-CA-17287 Aspects of Control in Mammalian Gene Expression
George Stark Stanford University

R01-CA-18065 Biogenesis of Messenger RNA in Animal Cells
Mary P. Edmonds University of Pittsburgh

R01-CA-20124 Messenger RNA of Normal and Malignant Human Cells
Grady F. Saunders University of Texas System Cancer Center

R01-CA-23226 Hybridizable Tumor Virus DNA in Cancer Tissues
Nelson Fausto Brown University

R01-CA-24165 Hemoglobin Studies in Friend Leukemia
Robert Zucker Papanicolaou Cancer Research Institute

R01-CA-24273 Expression of Globin Genes--Erythroleukemia Cells
Giovanni Rovera Wistar Institute of Anatomy and Biology

R01-CA-24635 Chromosomal Manifestations of Gene Expression
Peter W. Melera Sloan Kettering Institute for Cancer Research

R01-CA-25078 Poly(A) Polymerases from Liver and Hepatomas
Samson T. Jacob Pennsylvania State University

R01-CA-26073 Mapping Globin Sequences in Nuclear Ribonucleoprotein
Leonard H. Augenlicht Sloan Kettering Institute for Cancer Research

R01-CA-26790 Phenotypic Variation and Neoplastic Progression
Jerry Peterson Children's Hospital Medical Center Northern CA

R01-CA-27358 Messenger RNA Metabolism in Mammalian Cells
Ajit Kumar George Washington University

R01-CA-28555 Neoplastic Activation of Fetal Gene Expression
James A. Hoch Scripps Clinic and Research Foundation

R01-CA-29533 RNA Metabolism in Development and Hormonal Regulation
Joe L. Key University of Georgia

T-RNA

R01-CA-10922 Metabolism of Animal Cell Ribosomes
Maurice H. Vaughan University of Pittsburgh

R01-CA-20683 Control Mechanisms in Human Tumor Cells--Small RNAs
George L. Eliceiri St. Louis University

R01-CA-20919 tRNA Q-Base: Its Relation to Differentiation
Jon R. Katze University of Tennessee Center Health Science

R01-CA-21245 Structure and Modification of Mammalian Transfer RNA
Edward E. Penhoet University of California, Berkeley

R01-CA-23363 tRNA Methylation in Mammalian Cells
Phoebe S. Leboy University of Pennsylvania

R01-CA-23536 Molecular Biology of Ethionine Carcinogenesis
Ernest Borek AMC Cancer Research Center and Hospital

R01-CA-24727 Changes in RNA Metabolism and the Induction of Tumors
David Apirion Washington University

R01-CA-26423 Modification of tRNA 1/4YS Controls Cell Proliferation
Baryl J. Ortwerth University of Missouri

R01-CA-27235 tRNA Modification and Gene Expression in Mammalian Cells
Wesley G. Hatfield University of California, Irvine

R01-CA-27288 Regulation of Gene Expression in Human Tumor Cells
George L. Eliceiri St. Louis University

R01-CA-28053 Thiolated tRNAs in Rat Liver and Morris Hepatomas
Ting-Wa Wong University of Chicago

R01-CA-28395 tRNA Methylation in Normal and Neoplastic Rat Tissues
Phoebe Leboy University of Pennsylvania

DNA

R01-CA-11637 Control of DNA Replication in Normal and Tumor Cells
Peter Ove University of Pittsburgh

R01-CA-11981 Synthetic and Biological Studies of Nucleic Acids
Har G. Khorana Massachusetts Institute of Technology

R01-CA-14835 DNA Polymerases in Normal and Neoplastic Human Cells
David Korn Stanford University

R01-CA-15044 Pathogenetic Determinants of Human CNS Tumors
Laura M. Manuelidis Yale University

R01-CA-15187 DNA Synthesis: Regulation in Normal and Cancer Cells
Earl F. Baril Worcester Foundation for Experimental Biology

R01-CA-15915 The Structure and Function of a DNA Replicase
Neal C. Brown University of Massachusetts Medical School

R01-CA-16790 DNA Transcription Control in Normal and Cancer Cells
Joseph H. Maio Yeshiva University

R01-CA-17143 Characterization of Lymphocyte Excreted DNA
John C. Rogers Washington University

R01-CA-17723 Regulation of DNA Synthesis in the Novikoff Hepatoma
Ralph R. Meyer University of Cincinnati

R01-CA-18137 Persistence of Alkylated DNA in Carcinogenesis
Anthony E. Pegg Pennsylvania State University

R01-CA-18138 Mammalian Polyamine Metabolism
Anthony E. Pegg Pennsylvania State University

R01-CA-18543 DNAses and Leukemia
Jay S. Roth University of Connecticut

R01-CA-18564 Chediak-Higashi and Cancer Cells With Similar Defects
Janet M. Oliver University of Connecticut Health Center

R01-CA-18970 Reaction of Carcinogens and Cell Extracts with RF-DNA
William D. Taylor Pennsylvania State University

R01-CA-22610 Cytoskeleton and Cell Transformation to Malignancy
B. R. Brinkley Baylor College of Medicine

R01-CA-24151 Regulation of Cell Growth by 5'-Methylthioadenosine
Arthur A. Vandenbark University of Oregon Health Sciences Center

R01-CA-24158 DNA Synthesis in Transformed Cells
James M. Collins Virginia Commonwealth University

R01-CA-24283 Thymidine Metabolism at the Cell Surface
Phyllis R. Strauss Northeastern University

R01-CA-24323 DNA Precursor Dynamics in Animal Cells
Christopher K. Mathews Oregon State University

R01-CA-24845 The Fidelity of DNA Replication in Human Lymphocytes
Lawrence A. Loeb University of Washington

R01-CA-25023 Inhibitor of DNA Polymerase Produced by Tumor Cells
A. Arthur Gottlieb Tulane University

R01-CA-26360 Study of a Liver Helix Destabilizing Protein In Vivo
Gordhan L. Patel University of Georgia

R01-CA-26391 Molecular Pathology of Leukemia and Lymphoma
Mary S. Coleman University of Kentucky

GROWTH FACTORS

R01-CA-11176 Factors Required for Mammalian Cell Division
Robert W. Holley Salk Institute for Biological Studies

R01-CA-13808 Transformation and Recovery of Crown Gall Tumor Cells
Henry N. Wood Rockefeller University

R01-CA-14019 Tumor Angiogenesis: A Control Point in Tumor Growth
Moses J. Folkman Children's Hospital Medical Center

R01-CA-16748 Structure of Tumor Regulatory Proteins
Keith O. Hodgson Stanford University

R01-CA-17203 cAMP and the Membrane in Normal and Malignant Cells
Joseph T. Tupper Syracuse University

R01-CA-17620 Growth Control in Normal and Neoplastic Cells
Gary L. Smith University of Nebraska

R01-CA-19731 Hormonal Requirement of Cells In Vitro and In Vivo
Gordon H. Sato University of California, San Diego

R01-CA-21763 Growth Control by a Mitogen Purified from Cartilage
Michael Klagsbrun Children's Hospital Medical Center

R01-CA-22410 Copper Absorption and Ceruloplasmin in Cancer
Maria C. Linder California State University

R01-CA-23906 Tumor Necrosis Factor: Purification and Mode of Action
Saul Green Sloan Kettering Institute for Cancer Research

R01-CA-24395 Fibroblast Growth Factor and Phosphorylation
Marit Nilsen-Hamilton Salk Institute for Biological Studies

R01-CA-27031 Maturation Factors and Cancer Cells
Ramon Lim University of Chicago

R01-CA-27113 Molecular Analysis of Platelet-Derived Growth Factor
Charles D. Scher Sidney Farber Cancer Institute

R01-CA-27217 Growth Factors and Receptors in Chemical Transformation
Carol L. Moses Mayo Foundation

R23-CA-27802 Pharmacologic Modulation of Erythroid Colony Formation
Barbara G. Beckman Tulane University of Louisiana

R01-CA-28110 Nerve Growth Factor: Secretion by Cancer Cells
David M. Young University of Florida

R01-CA-28456 Biochemical Mechanisms of Cellular Invasion
Daniel B. Rifkin New York University

R01-CA-28540 Growth and Migration of Capillary Endothelial Cells
Bruce R. Zetter Children's Hospital Medical Center

R01-CA-28638 Altered Nutritional Requirements for Growth
William C. Topp Cold Spring Harbor Laboratory

R01-CA-28858 Biological and Synthetic Modulators of Cell Growth
Loren R. Pickart Virginia Mason Research Center

NUCLEUS

R01-CA-10871 Structure and Function of Chromosomal Nucleohistone
Roger G. Chalkley University of Iowa

R01-CA-10872 Interactions of Chromosomal Proteins
Irvin Isenberg Oregon State University

R01-CA-12226 Protein Methylation in Neoplastic Tissues
Woon K. Paik Temple University

R01-CA-12877 Properties and Function of Nuclear Phosphoproteins
Thomas Langan University of Colorado Medical Center

R01-CA-13195 Histone ADP-Ribosylation and HeLa Cell Replication
Marc E. Smulson Georgetown University

R01-CA-13196 Group Transfer Processes in Normal and Malignant Cells
Roberts A. Smith University of California, Los Angeles

R01-CA-15135 Histones in Cell Differentiation and Carcinogenesis
Alfred Zweidler Fox Chase Cancer Center

R01-CA-15923 Nonhistone Proteins of Reactivated Erythrocyte Nuclei
Keith R. Shelton Virginia Commonwealth University

R01-CA-16910 Chromosome Aberrations in Cancer
Janet D. Rowley University of Chicago

R01-CA-17782 Tumor-Enriched Nonhistone Chromatin Proteins
Gerald R. Reeck Kansas State University

R01-CA-17856 Regulation of Histone Biosynthesis in Heteroploid Cells
Peter J. Curtis Wistar Institute of Anatomy and Biology

R01-CA-18389 Proteins of the Cell Nucleus
Lubomir S. Hnilica Vanderbilt University

R01-CA-18455 Isolated Chromosomes in Genetics and Cancer Research
Granville W. Wray Baylor College of Medicine

R01-CA-21927 Chromatin Structure of Normal and Malignant T-Cells
Abby L. Maizel University of Texas System Cancer Center

R01-CA-22302 Analysis of Gene Regulation by Nuclear Transplantation
Joseph J. Lucas State University of New York

R01-CA-24546 Relation of Histones to DNA in Normal and Cancer Cells
Roger D. Kornberg Stanford University

R01-CA-25055 Cytogenetics of Clonal Neoplasias
Frederick Hecht Southwest Biomedical Research Institute

R01-CA-25735 Regulatory Processes Biochemistry in Synchronized Cells
Lon D. Hodge Medical College of Georgia

R01-CA-27446 Nuclear Proteins in Hepatoma and Normal Liver
Erkki I. Ruoslahti La Jolla Cancer Research Foundation

R01-CA-28168 Cytogenetics of Hepatocellular Carcinoma in Nigeria
Jaroslav Cervenka University of Minnesota

R01-CA-28679 Chromosomal Organization of Dihydrofolate Reductase Gene
June L. Biedler Sloan Kettering Institute for Cancer Research

CONTRACTILE ELEMENTS

R01-CA-05493 Leukopoietic Mechanisms
Peter P. De Bruyn University of Chicago

R01-CA-15229 Actin & Tubulin Cancer Cells (Human, Adenovirus)
Robert R. Weihing Worcester Foundation for Experimental Biology

R01-CA-15544 Effect of Microtubular Proteins on Cell Surfaces
Richard D. Berlin University of Connecticut Health Center

R01-CA-17210 Normal and Tumor-Virus Transformed Cell Behavior
Robert D. Goldman Carnegie-Mellon University

R01-CA-20836 Cytochalasins and Cell Membrane Contractile Apparatus
Gabriel C. Godman Columbia University

R01-CA-26473 ACTH Effects on the Cytoskeleton of Adrenal Tumor Cells
Priscilla Mattson Case Western Reserve University

R01-CA-26867 Invasion and Metastasis
Irving Zeidman University of Pennsylvania

R01-CA-27458 Biochemistry of Cytoskeletal Proteins
George P. Tuszynski Temple University

R01-CA-28362 Levels of Metastasis Inhibition in Primary Cancers
Dorothy G. Rapp Roswell Park Memorial Institute

R01-CA-29551 Complement Mediated Tumor Cell Chemotaxis
Peter A. Ward University of Michigan

DEVELOPMENT AND DIFFERENTIATION

R01-CA-02662 Investigations on Teratocarcinogenesis
Le Roy C. Stevens Jackson Laboratory

R01-CA-08482 Differentiation and Function of Hematopoietic Cells
Robert B. Scott Virginia Commonwealth University

R01-CA-10095 Gene Action and Cellular Differentiation in Culture
Selma Silagi Cornell University Medical Center

R01-CA-11339 Control of Cell Growth, Differentiation, and Function
Charity Waymouth Jackson Laboratory

R01-CA-11689 Differentiation and Stroma-Induction in Neural Tumors
Lucien J. Rubinstein Stanford University

R01-CA-13047 Control Mechanisms of Differentiation and Malignancy
Charlotte Friend Mount Sinai School of Medicine

R01-CA-13533 Ectopic Placental Proteins in Human Cancer
Howard H. Sussman Stanford University

R01-CA-14319 Biological Functions of Plasma Membrane Sialidase
Cara L. Schengrund Pennsylvania State University

R01-CA-15222 Hepatoma Alpha-Fetoprotein: Chemistry and Metabolism
Carol J. Woodworth University of Vermont & State Agricultural College

R01-CA-16368 Control of Differentiation of Erythroleukemic Cells
Arthur I. Skoultchi Yeshiva University

R01-CA-16746 Control of Albumin Synthesis in Liver and Hepatomas
John M. Taylor Pennsylvania State University

R01-CA-17563 DNA-Binding Proteins in Human Serum
Sallie O. Hoch Scripps Clinic and Research Foundation

R01-CA-17575 Erythroid Differentiation in Friend Leukemia Cells
David E. Housman Massachusetts Institute of Technology

R01-CA-18314 Leukemia Cell Systems: Induction of Differentiation
Paul A. Marks Columbia University

R01-CA-18375 Hemopoietic Stem Cells and Induced Differentiation
Eugene Goldwasser University of Chicago

R01-CA-19492 Terminal Transferase in Mammalian Hemopoietic Cells
Mary S. Coleman University of Kentucky

R01-CA-20053 Heritable Changes in Plant Tumor Development
Frederick Meins University of Illinois

R01-CA-21246 Studies of Mouse Teratoma and Derived Cells
Albert E. Chung University of Pittsburgh

R01-CA-21566 Antitumor Invasion Factors Derived from Cartilage
Klaus E. Kuettner Rush-Presbyterian-St. Lukes Medical Center

R01-CA-21602 Biochemical Events in Differentiating Neuroblastoma
Roger N. Rosenberg University of Texas Health Science Center

R01-CA-22227 Onco-Developmental Gene Control: Alpha-Fetoprotein
Stewart Sell University of California, San Diego

R01-CA-22294 Quantitative Studies on Granulocyte Differentiation
Joseph M. Kinkade Emory University

R01-CA-22556 Differentiation of Granulocytes and Monocytes
Donald Metcalf University of Melbourne

R01-CA-22558 Comparative Biochemistry of Cartilage Tumors
Theodore R. Oegema University of Minnesota

R01-CA-22670 Control of Hemopoietic Stem Cell Differentiation
John J. Trentin Baylor College of Medicine

R01-CA-22735 Thymic Modification of Leukemogenesis
Lyle Sensenbrenner Johns Hopkins University

R01-CA-22815 Studies in Neuro-Oncogenesis and Neurogenesis
Ian S. Zagon Pennsylvania State University

R01-CA-23097 Embryo Derived Teratocarcinoma
Ivan Damjanov Hahnemann Medical College & Hospital

R01-CA-23357 Ectopic HCG-Beta from Pure Beta-Producing Cells
Robert O. Hussa Medical College of Wisconsin

R01-CA-23528 Inhibitory Interactions Regulating Hematopoiesis
Hal E. Broxmeyer Sloan Kettering Institute for Cancer Research

R01-CA-23533 Tumor Immunology; Phosphatase Isoenzymes
George J. Doellgast Wake Forest University

R01-CA-23615 Molecular Basis of Differentiation and Neoplasia
Robert G. Roeder Washington University

R01-CA-23985 The Development and Pathology of Human Choriocarcinoma
Susan J. Friedman University of Colorado Medical Center

R01-CA-24010 Differentiation of Cancer
Barry Pierce University of Colorado Medical Center

R01-CA-24206 Embryonal Carcinoma Growth and Differentiation
Elwood A. Linney La Jolla Cancer Research Foundation

R23-CA-24394 Murine Erythroleukemia Cells Induced to Differentiate
Roberta C. Reuben Columbia University

R01-CA-24402 Molecular Control of Friend Leukemia Cell Maturation
William Scher Mount Sinai School of Medicine

R01-CA-24479 Membrane Structure & Enzyme Induction
Kuang Yu Chen Rutgers, The State University

R01-CA-25098 Alpha-Fetoprotein Regulation in Fetal & Cancer Liver
Jen-Fu Chiu University of Vermont & State Agricultural College

R01-CA-25164 Nucleocytoplasmic Regulation of Malignant Phenotypes
Oscar Sudilovsky Case Western Reserve University

R23-CA-25285 Comparative Biology of Trophoblast and Tumor
Paul Higgins Sloan Kettering Institute for Cancer Research

R01-CA-25512 Modulators of Granulopoiesis from Human Cell Lines
James K. Brennan University of Rochester

R01-CA-25966 X-Chromosome Activity in Teratocarcinoma Stem Cells
Gail R. Martin University of California, San Francisco

R01-CA-25972 Leukemia Induction in Multipotential In Vitro Colonies
Donald Metcalf Walter and Eliza Hall Institute

R01-CA-25985 Response of Phagocytic Leukocytes to Tumor Promotors
Judith K. Christman Mount Sinai School of Medicine

R01-CA-26014 Cell Interactions in Tetratocarcinoma Differentiation
Maurice J. Rosenstraus Rutgers, The State University

R01-CA-26038 Study of Myeloid Leukemia Using Human Leukemia Lines
H. Phillip Koeffler University of California, Los Angeles

R01-CA-26105 Human Renal Cancer and Hematopoiesis
H. Phillip Sytkowski Children's Hospital Medical Center

R01-CA-26234 Fibroblastic Colonies in Myeloproliferative Disorders
Bernard R. Greenberg University of California, Davis

R23-CA-26401 Human Leukemia Marrow
Joseph W. Fay Duke University

R01-CA-26506 Myeloid Development in an Induced Leukemic Cell Line
Harvey J. Cohen Children's Hospital Medical Center

R01-CA-26656 Cell Culture Analysis of Human Epidermal Neoplasia
James G. Rheinwald Sidney Farber Cancer Institute

R01-CA-26836 Surface Chemistry of Teratocarcinoma and Embryos
Marie F. Maylie-Pfenning Columbia University

R01-CA-26847 Regulation of mRNA Translation in Animal Cells
Lee A. Weber University of South Florida

R01-CA-26993 Cytotoxic Factor(s) from Human Ovarian Adenocarcinoma
Bertrum Sheid Downstate Medical Center

R01-CA-27287 Vaginal and Cervical Epithelial Cell Culture Model
Robert H. Rice Harvard University

R01-CA-27464 The Placenta Specific Protein, SP1, in Cancer
Eva S. Engvall La Jolla Cancer Research Foundation

R01-CA-27466 Endothelial Colony-Stimulating Activity
Peter J. Quesenberry University of Virginia

R01-CA-27580 Chromatin Proteins of Embryonal Carcinoma Cells
Robert G. Oshima Massachusetts Institute of Technology

R01-CA-27682 New Technology for Classifying Human Leukemia
Howard D. Stang University of California, Los Angeles

R01-CA-28050 Regulation of Alpha-Fetoprotein Gene Expression
Shirley M. Tilghman Institute for Cancer Research

R01-CA-28106 Differentiation of Teratocarcinoma Cells
Arnold J. Levine State University of New York

R01-CA-28179 Lithium Effects on Hemopoietic Stem Cells
Robert A. Joyce University of Pittsburgh

R01-CA-28228 Expansion of Hemopoietic Bone Marrow
Minako Y. Lee University of Washington

R01-CA-28427 EGF and Its Receptors in Embryonic Differentiation
Eileen D. Adamson La Jolla Cancer Research Foundation

R23-CA-28512 Regulation of Normal and Leukemic Myeloid Stem Cells
Louis M. Pelus Sloan Kettering Institute for Cancer Research

R01-CA-28815 Biosynthesis of Carcinoembryonic Antigen
Joseph F. Mendicino University of Georgia

CELL GROWTH

R01-CA-06663 Repair of Chromatin by 3-Methyladenine N-Glycosylase
Irving Lieberman University of Pittsburgh

R01-CA-08373 Study of Factors Controlling Cellular Proliferation
Renato L. Baserga Temple University

R01-CA-12187 Growth and Differentiated Function in Mammalian Cells
Howard Green Massachusetts Institute of Technology

R01-CA-14525 Biochemistry of Normal and Leukemic Lymphocytes
Creed W. Abell University of Texas Medical Branch

R01-CA-15305 Effect of Malignancy on Cell Growth Requirements
Richard Ham University of Colorado

R01-CA-15381 Control of Tumor-Induced Vascularization
Alan Fenselau Johns Hopkins University

R01-CA-15744 Primary Events in Regulating Cell Multiplication
Harry Rubin University of California, Berkeley

R01-CA-15813 Lipid Transport and Metabolism in Cancer-Host Systems
Nome Baker University of California, Los Angeles

R01-CA-16463 Triiodothyronine Receptors and Thyrotroph Neoplasia
Martin I. Surks Montefiore Hospital and Medical Center

R01-CA-17283 Lipid Metabolism of Leukocytes
Charles P. Burns University of Iowa

R01-CA-17650 Control of Cell Division in Normal vs Malignant Cells
Leonard J. Bello University of Pennsylvania

R01-CA-19126 Cystine Auxotrophy in Human Malignancy--Implications
Herbert Lazarus Sidney Farber Cancer Institute

R01-CA-20136 Tumor Lipids: Metabolism and Structural Studies
Randall Wood Texas Agricultural and Mechanical University

R01-CA-20709 Cellular Incorporation of Vitamin B-12
Richard A. Gams University of Alabama

R01-CA-21359 Cell Interactions During Malignant Transformation
John S. Bertram Roswell Park Memorial Institute

R01-CA-22011 Studies of Myeloid Leukemia in RFM Mice
Frank E. Trobaugh Rush-Presbyterian-St. Lukes Medical Center

R01-CA-22042 Role of Serum in the Foreign Body Response
Charles Stiles Sidney Farber Cancer Institute

R01-CA-22088 Control of Growth in Quiescent Human Cells
Allen J. Norin Montefiore Hospital and Medical Center

R01-CA-23022 Studies of Mitosis in Normal and Neoplastic Cells
B. R. Brinkley Baylor College of Medicine

R01-CA-23878 Role of Polyamines in Mammalian Cell Proliferation
Potu N. Rao University of Texas System Cancer Center

R01-CA-24193 Regulation of Mammalian Cell Cycle
Warren J. Pledger University of North Carolina

R01-CA-24914 Control of Cell Division in Leukemia Cells
R. Michael Wolcott Louisiana State University Medical Center

R01-CA-24961 Protein Synthesis and Control of the Cell Cycle
Laroy N. Castor Institute for Cancer Research

R01-CA-25009 Role of Disulfides in Normal and Tumor Cell Growth
Robert C. Fahey University of California, San Diego

R01-CA-26070 Control of Cycle Progression in Animal Cells
Claudio Basilico New York University

R01-CA-26081 Cell Cycle Dependence of Cell Surface Receptors
Janos M. Varga Yale University

R23-CA-26889 Regulation of Cell Growth by Membrane Ion Flux
Christopher N. Frantz Sidney Farber Cancer Institute

R23-CA-26956 Detailed Cell Kinetic Analyses of Human Neuroblastoma
John E. Neely Children's Hospital Medical Center

R01-CA-27151 Regulation of Cell Replication by Thymidine
Harvard Reiter University of Illinois Medical Center

R01-CA-27399 Regulation of Mitosis in Normal and Transformed Cells
Jesse E. Sisken University of Kentucky

R01-CA-27419 Immunopharmacologic Consequences of Tumor Heterogeneity
Gloria H. Heppner Michigan Cancer Foundation

R01-CA-27544 Purification and Characterization of Mitotic Factors
Sai P. Rao University of Texas System Cancer Center

R01-CA-27562 Regulation of Transfer RNA Levels in Mammalian Cells
Michael Litt University of Oregon Health Sciences Center

R01-CA-27564 Methionine Dependence--A Metabolic Marker in Cancer
Robert M. Hoffman University of California, San Diego

R23-CA-27808 Plasticity of Chromaffin and Pheochromocytoma Cells
Arthur S. Tischler Tufts University

R01-CA-27809 Pathways of Energy Metabolism in Malignancy In Vivo
Leonard A. Sauer Mary Imogene Bassett Hospital

R01-CA-27923 Aortic Growth Inhibitors
Reuben Eisenstein Northwestern University

R01-CA-28140 A Novel Vitamin B6 Metabolite in Hepatomas
George P. Tryfiates West Virginia University

R01-CA-28238 Effects of Mitogens on Normal and Neoplastic Cells
Arthur M. Vogel University of Washington

R01-CA-28240 Pathology in Cell Cycle Control of Differentiation
Robert E. Scott Mayo Foundation

R23-CA-28329 Phase Specific DNA Repair in Irradiated Tumor Cells
Peter C. Keng University of Rochester

R01-CA-28964 Tumor Cell-Vascular Endothelial Cell Interactions
Charles R. Birdwell La Jolla Cancer Research Foundation

R01-CA-29560 Cyclic AMP Analogs as Growth Regulators in Tumor Cells
Wesley D. Wicks University of Tennessee

SOMATIC CELL GENETICS

R01-CA-12130 Cytoplasmic Inheritance in Normal and Tumor Cells
Morgan Harris University of California, Berkeley

R01-CA-16631 Isolation and Analysis of DNA Mutants of BHK Cells
Harriet K. Meiss New York University

R01-CA-16720 Gene Regulation & Interaction--Normal & Malignant Cells
Harold P. Klinger Yeshiva University

R01-CA-16754 Hybridization, DNA Function, Mutation in Cell Culture
John W. Littlefield Johns Hopkins University

R01-CA-19401 Genetic Analysis of Human Malignancy
Eric J. Stanbridge University of California, Irvine

R01-CA-21054 Genetic Analysis of Malignant Transformation
Seung-Li Shin Yeshiva University

R01-CA-23003 Regulation of Expression of Viral Transformation
Harvey L. Ozer Hunter College

R01-CA-24828 Genetic Analysis of Tumorigenesis
Ruth Sager Sidney Farber Cancer Institute

R01-CA-25342 Complementation Studies for SCE With Cell Hybrids
Marcello Siniscalco Sloan Kettering Institute for Cancer Research

P01-CA-25875 Cell Differentiation and Cancer
Carlo M. Croce Wistar Institute of Anatomy and Biology

R01-CA-27607 Coordinated Control of Mammalian Gene Expression
Amy S. Lee University of Southern California

R01-CA-27712 Gene Expression During Mammalian Development
Carlo M. Croce Wistar Institute of Anatomy and Biology

R01-CA-27713 Gene Expression During Mammalian Development
Karl M. Illmensee University of Geneva

INHERITANCE OF NEOPLASMS

R01-CA-12643 Genetically-Controlled Malignant Neoplasms
Howard A. Bryant University of California, Irvine

PLASMIDS, VIRUSES

R01-CA-11526 Tumor-Inducing Substance of Agrobacterium tumefaciens
Clarence I. Kado University of California, Davis

R01-CA-13015 Molecular Basis of Crown Gall Tumorigenesis
Eugene W. Nester University of Washington

R01-CA-14026 Tumor Virus, Plasmid & Cell Analysis
Howard Goodman University of California, San Francisco

R01-CA-18604 The Mechanism of Tumorigenesis by A. tumefaciens
Ann G. Matthysse University of North Carolina

R01-CA-19402 Molecular Genetics of Agrobacterium Plasmids
Stephen K. Farrand Loyola University Medical Center

R23-CA-27421 Development of a Eukaryotic Cloning Vehicle
Cristina M. Rogers Oregon State University

R01-CA-27424 Agrobacterium DNA Insertion and Expression in Plants
Howard M. Goodman University of California, San Francisco

IN VIVO AND IN VITRO TUMOR LINES

R01-CA-17229 Keloids: An In Vitro Model of Tumor Growth Regulation
James D. Russell Meharry Medical College

R23-CA-23871 Social Behavior of Cultured Colon Adenocarcinoma Cells
Lynne P. Rutzky University of Texas Health Science Center

R01-CA-24145 Ovarian Tumors in Young Mice
Wesley K. Beamer Jackson Laboratory

R23-CA-24393 Derivation of Nerve Cell Lines from the Brain
Gregory J. Giotta Salk Institute for Biological Studies

R01-CA-24621 Induction-Continuation-Genetics of Experimental Tumor
Harold Morris Howard University

R01-CA-25630 Studies of Malignant Progression Using Human Cells
Helene S. Smith University of California, Berkeley

R01-CA-25718 Swine Melanoma: In Vitro Growth and In Vivo Pathology
Jane Berkelhammer Cancer Research Center

R01-CA-26063 Primary Culture of Prostatic Epithelial Cells
William H. Douglas Tufts University

R01-CA-26110 Androgen-Responsive Prostate Epithelial Cells
Wallace L. McKeehan W. Alton Jones Cell Science Center

R01-CA-28641 Tumor Imaging Using ^{99m}Tc and ^{111}In-Labeled Leukocytes
Geraldine Dettman Brown University

R01-CA-28668 Biologic Markers for Melanoma
Charles W. Gehrke University of Missouri

R01-CA-29550 Tumor Cell With Varying Degrees of Malignancy
James Varani University of Michigan

CONFERENCES

R13-CA-15961 Seminars and Workshops in Techniques of Cancer Research
Donald W. King University of Colorado Medical Center

R23-CA-24071 Receptors for Epidermal Growth Factor in Tumor Cells
Graham F. Carpenter Vanderbilt University

R13-CA-26729 1980 Annual Symposium on Fundamental Cancer Research
Charles A. Le Maistre University of Texas System Cancer Center

R13-CA-28088 International Forum: Cancer-Cell Organelles
James D. Morre Purdue University

R13-CA-28908 Gordon Research Conference on Cancer, 1980
Frederick F. Becker Gordon Research Conferences

PROGRAM PROJECTS

P01-CA-10893 Cancer Research Center
Harris Busch Baylor College of Medicine

P01-CA-12174 Study of Growth and Cell Control Processes
Howard Green Massachusetts Institute of Technology

P01-CA-12923 A Correlated Study on the Biology of Neoplasia
Renato L. Baserga Temple University

P01-CA-14294 Tumor Associated Changes of Intestinal Cells
Kurt J. Isselbacher Massachusetts General Hospital

P01-CA-14454 Membranes in Normal and Cancer Cells
Ephraim Racker Cornell University

P01-CA-15823 Program in Developmental Biology of Cancer
Barry G. Pierce University of Colorado Medical Center

P01-CA-19265 Cancer Biology Research Center
John E. Ultmann University of Chicago

P01-CA-20810 Somatic Cell Genetics in Cancer
Theodore T. Puck University of Colorado

P01-CA-21069 Cell Biology and Immunology of the Cancer Cell
Leonard Warren Wistar Institute of Anatomy and Biology

P01-CA-21901 Studies of Normal and Malignant Cell Membranes
Saul Roseman Johns Hopkins University

P01-CA-22376 Control of Gene Expression: Normal and Neoplastic
Phillip Feigelson Columbia University

P01-CA-23052 Program Project on Athymic Mice and Human Tumors
Nathan O. Kaplan University of California, San Diego

P01-CA-23076 Regulatory Mechanisms in Tumor Biology
Gerald C. Mueller University of Wisconsin

P01-CA-25845 Pathobiology of Small Cell Carcinoma of the Lung
George Sorenson Dartmouth College

P01-CA-26712 Glycoproteins, The Cytoskeleton and Cancer
Richard O. Hynes Massachusetts Institute of Technology

DIFFICULT TO CLASSIFY

R01-CA-09247 Partial Subsidy for the Journal of Cancer Research
Frederic S. Philips American Association for Cancer Research

R01-CA-21607 Secretory Processes in the Prostate Gland
Emil R. Smith University of Massachusetts Medical School

R01-CA-27120 Interferon System: Action, Induction, and Regulation
Paul O. Ts'o Johns Hopkins University

R01-CA-28571 Recognition of Patterns in Cancer-Related Sequences
Bruce W. Erickson Rockefeller University

IMMUNOLOGY

The Immunology Program of the Division of Cancer Biology and Diagnosis is composed of three sections with focus on Molecular Immunology, Cellular Immunology and Clinical Immunology. Support is provided for a spectrum of research which reflects the need for more information on basic aspects of the immune system as well as the importance of translating that information to studies on animal tumors as models and ultimately to the clinic and human cancer. The areas of this support can be defined by reference to the referral guidelines for the program.

The Immunology Program of the National Cancer Institute supports investigations on the effects of the immune system upon the initiation, development, growth, spread and control of tumors.

Specific areas include:

1) The synthesis and structure of myeloma proteins in animals and man.

2) The synthesis, structure and function of antibodies capable of reacting with tumor cells, agents which induce tumors and agents used in the treatment of tumors.

3) The synthesis, structure and function of humoral factors other than antibody which participate in, activate and/or regulate the immune response to tumors. This would include complement, interferon, lymphokines, lymphoid cell growth factors, helper factors, suppressor factors, etc., as they are involved in immune responses to tumors.

4) The immunobiology of lymphocytes which participate in anti-tumor responses including their development, heterogeneity, interactions and functions.

5) The immunobiology of malignancies of the immune system (lymphomas and leukemias) including studies of immunologic markers for the classification and characterization of neoplastic cells and their normal counterparts.

6) The immunobiology of monocytes and macrophages which participate in anti-tumor responses including their development, heterogeneity, interactions and functions.

7) The identification, isolation and characterization of cell surface determinants which serve as target antigens for the immune response to tumors (e.g., tumor specific antigens, tumor associated antigens).

8) The identification, isolation and characterization of cell surface determinants of lymphocytes and macrophages which are involved in the responses of these cells to tumors.

9) Immune surveillance against the development of tumors of various origins by all immune mechanisms (e.g. T cell immunity, macrophage reactivity, natural killer cell activity).

10) Immunopathology studies on the host-tumor interaction.

11) Immune status of tumor bearing animals and man including studies on immunostimulation, immunosuppression, and the effects of disease course on immune function.

12) Bone marrow transplantation in man and animals as a treatment for cancer when the emphasis is on immunologic problems such as immune reconstitution, sensitization and graft versus host disease.

13) Immunotherapy in animal models including studies on specific and non-specific stimulation of the immune system using natural and synthetic agents.

14) Immunotherapy including pre-clinical and clinical protocols where the main emphasis is upon the study of immune parameters, immune mechanisms and other immunologic concerns rather than upon a therapeutic result. Included are studies on specific and non-specific stimulation of the immune system using natural and synthetic agents.

15) Immunobiology of sarcomas, carcinomas and melanomas.

Studies which utilize immunological techniques but which are not directed at the involvement of the immune system in the host-tumor relationship can be found in other programs (e.g., immunodiagnosis is included in the Diagnosis Program, DCBD).

For fiscal year 1980, the program provides $58.7 million of support in grant programs with this consisting of approximately $41 million in R01 grants and $17.7 million in P01 grants. Support of $3.4 million to research and resources was provided by the contract mechanism during fiscal year 1980.

The following examples of research supported during fiscal year 1980 are chosen to reflect the broad content of the program and to illustrate how the basic and applied areas of the program are synergistic with each other. The importance of maintaining support of a balanced yet comprehensive immunological research program to meaningful progress is evident in such examples.

A. Studies on B Cells:

The immune system is of interest not only for the role it plays in host defense against tumors but also because it is itself often the target of oncogenic transformation. This duality provides a context for cross fertilization between basic and clinical studies which has been very productive. As an example of this, one can consider some of the recent work on one major subclass of lymphocytes - B cells (bursal-equivalent derived). In humans, tumors with the characteristics of B cells are a significant clinical problem and include myelomas, plasma cell tumors, most cases of non-Hodgkin's lymphoma and chronic lymphocytic leukemia and a small proportion of cases of acute lymphocytic leukemia.

Over the past several years, the study of murine and human plasma cell tumors has provided extensive information on the synthesis, secretion and structure of immunoglobulin molecules. While further studies on these molecules and on the organization, regulation and expression of the genes coding for immunoglobulin remain of great interest, these B cell tumors have been very useful for other studies as well.

For example, a recent study in Eisen's laboratory (Hale, et al., 1979; Celis, et al., 1979) has used a murine myeloma to explore cyclical changes in the susceptibility of a tumor to immune destruction. Myeloma cells transplanted to a syngeneic host could be recovered after a short period and were susceptible to both cell and antibody-mediated cytotoxicity in an assay where products of the major histocompatibility locus were the target antigens. However, if the tumor cells were given a longer period of residence in the host, the tumor could still be easily recovered but the cells were no longer susceptible to immune killing. This resistance was associated with a quantitative increase of a particular cell surface glycoprotein, and removal of this molecule from the cell membrane by enzymatic digestion restored susceptibility to immune lysis. The importance of such a study is in what it reveals concerning why tumors are not eliminated by the immune system and how this might be circumvented. By demonstrating that changes in antigenic expression can occur within individual cells as well as by selection of pre-existing variants, it heightens our appreciation of the dynamic nature of the host-tumor relationship and suggests operative mechanisms for a therapeutic strategy.

Another significant use of myeloma tumor cells is in somatic cell hybridization for the production of hybridomas capable of producing large amounts of monoclonal antibody. This hybridization technique was first described by Kohler and Milstein in England and has rapidly spread through the research community. A major element in facilitating this spread was (and is) the availability of appropriate drug-marked myeloma cells from a Cell Bank and Distribution Center maintained by M. Cohn at the Salk Institute and supported by an NCI Immunology Program contract. A major advantage of hybridoma produced monoclonal antibody over conventional antisera is a reduced degree of unwanted cross reactivity, thus leading to increased specificity and sensitivity. For example the search for tumor specific antigens using conventional antisera has been fraught with difficulties traceable to cross reactivity of these sera with

non-tumor antigens. However, use of monoclonal antibodies is already proving useful in this search. Steplewski and colleagues (Steplewski, et al., 1979) have reported on several hybridoma clones producing monoclonal antibodies which bind to melanoma cells but not to normal fibroblasts nor to cells from a giant hairy nevus of the same patient. The reactivity studied appears to be directed to an antigen(s) common to several melanoma cases. Work of a similar tact is proceeding in the Hellstroms' laboratory in Seattle (Yeh, et al., 1979). They have reported on several hybridoma clones which produce monoclonal antibody reacting strongly to the single melanoma line used for immunization, weakly to a small number of other melanoma lines, and not at all to other tumors or to normal fibroblasts. Additional characterization of the antibodies and the recognized antigens is in progress in both laboratories and a widespread application to clinical diagnosis and eventually to clinical therapy is possible. While these illustrative examples are with melanoma, other tumors such as colorectal cancer (Herlyn, et. al., 1979) and leukemia (Reinberg, et al., 1979) are equally amenable to such an approach and are receiving considerable attention.

A unique application of hybridoma technology (and thus use of myeloma cells) which is providing important information on a basic problem is found in the studies of Cancro, et al., (1979). They use somatic cell hybridization to analyze the diversity of the B cell repertoire by preserving individual antibody-specific lymphocyte clones as hybridomas. By doing so, sufficient secreted product from each clone is available for analysis. They found that neonatal mice of the Balb/c strain have a much more limited number of clonotypes than adults of the same strain (about 10% of the adult number). Further, all neonatal mice analyzed appeared to express the same clonotypes and this would indicate that the acquisition of clonal diversity is highly patterned and genetically determined. The significance of this finding is that it suggests a genetically programmed acquisition of repertoire rather than one dominated by random somatic events. Further studies in this system should be equally revealing of the process by which the immune system is capable of recognizing as many as 10^7 different antigens which has been a problem of key interest for biologists for some time.

A second new technology has developed simultaneously with somatic cell hybridization which is also having a significant impact upon tumor immunology. This is fluorescent activated cell sorting (FACS). Pioneered by Herzenberg at Stanford, this technique is capable of simultaneously analyzing several cellular parameters by the use of fluorescent probes. In immunological research, a fluorescein conjugated antibody to a cell surface component is most often used and one can quickly count the number of cells with that surface antigen, measure the amount of antigen present and physically separate cells differing in antigen expression. By coupling this technology with the use of various B cell tumors, Warner, et al., (1979) have made important progress in analyzing B cell differentiation. Tumor cells were categorized on the basis of cell surface immunoglobulin isotype and amount, membrane expression of Ia, Ly-b, plasma cell alloantigens and Fc receptors, and response to stimulatory agents. The premise of such studies is that each lymphoid tumor may represent a monoclonal neoplastic proliferation of a cell belonging to a particular B lymphocyte sub-population and may be arrested

at that stage of development. The tumors then can be used as relatively homogeneous populations to study the characteristics and function of a particular development stage thereby contributing to our basic understanding of immune processes. In addition, we gain information useful in more accurate diagnosis and in studying the natural history of lymphoid neoplasms.

Further illustration of the use of model B cell tumors and florescent activated cell sorting can be found in work with the murine lymphoma designated BCL-1. This tumor has been studied by Strober and colleagues at Stanford and by Vitteta and her colleagues in Dallas. After its spontaneous developmenmt in a Balb/c mouse, the tumor was shown to have the characteristics of an immature B cell in that cells show large amounts of surface IgM but very little IgD, Fc receptors and Ia antigens, no I-A antigens and does not secrete immunoglobulin. By phenotype, growth pattern and surface distribution (Krolick, et al., 1979) as established using the FACS, this tumor appears to closely parallel a rare form of human CLL by showing a late but pronounced leukemia, absence of solid tumor masses, spenomegaly, absence of lymph node involvement and late infiltration of the bone marrow. It appears that the tumor cells require the splenic microenvironment for growth and that the immature B cell tumor can be caused to further differentiate and secrete immunoglobulin by stimulation with bacterial lipopolysaccharide (Knapp, et. al., 1979). The use of various agents to control tumor growth by inducing maturation is an area receiving increased attention.

Moving from the basic studies, clinical application of cell sorting to B cell tumors in order to detect minimal disease is being studied in several laboratories (Ault, 1979; Ligler, et. al., 1979) and the technique appears to be more sensitive than alternate tests and therefore to offer a distinct advantage. B cell neoplasms are particularly amenable to such analysis as the idiotypic determinant(s) of the cell surface immunoglobulin can be used to define the malignant clone. Kubagawa, et. al., (1979) were able to use fluorescent staining with anti-idiotyopic antibody to examine a human neoplastic B cell clone. While the clinical presentation was of an IgA myeloma, increased numbers of cells in that specific clone were evident even at the pre-B cell maturational stage. A similar finding is evident in the study of human CML by Le Bien and coworkers (1979). These results may reflect an aberrant expression of feedback control due to the existence of large numbers of more mature cells but may alternatively indicate that the cell type found in largest numbers rather than itself being transformed may be several differentiative steps removed from the actual oncogenic transformation. Clearly, additional work in this area will be useful in understanding the biology of lymphoid tumors.

B. Bone Marrow Transplantation:

The role of allogeneic bone marrow transplantation as a therapeutic option continues to expand and broader application appears feasible. While marrow grafting has become the therapy of choice for severe aplastic anemia and combined immunodeficiency diseases and has provided encouraging results in acute leukemia, nevertheless, a major problem of recurrence of leukemia

continues to complicate this latter condition. Since the marrow transplant regimen coud result in prolonged remission and even cure some patients with end-stage disease, it was logical to utilize this approach earlier in the course of the disease. The Seattle team has reported favorable results in 22 patients with ALL in second or subsequent remission (Thomas et al., (1979) and in 19 patients with AML in first remission (Thomas et al., 1979). In the latter study twelve (12) patients are alive in remission at the present time, 13-34 months after marrow grafting. Only one patient had a relapse and died of leukemia. The median survival of this group of patients will not be less than nineteen months which compares favorably with the best reported results of chemotherapy in acute nonlymphoblastic leukemia even though no maintenance chemotherapy was given. Recent evidence suggests that patients with chronic myelocytic leukemia (CML) may also benefit from marrow grafting. Fefer et al., (1979) studied four (4) patients with Philadelphia chromosome (Ph^1) positive (CML) while they were in the chronic phase of the disease, who were treated with vigorous chemotherapy and radiation plus marrow from their normal identical twins.

These four patients are alive and well 24-40 months after grafting. They remain Ph^1 negative indicating a reasonable probability that the disease has been cured. The results justify testing the same approach using marrow from a matched sibling rather than from a twin.

Several recent observations and developments suggest even wider application for bone marrow transplantation in the coming decade. Most patients do not have HLA identical siblings and very few have monozygous (identical) twins. To extend marrow transplantation to a larger number of patients, the use of donors other than HLA identical siblings must be explored. A number of successful cases have now been reported by Dupont et al., (1979). One strategy to solve the problem of recurrent leukemia following bone marrow transplantation would be to transplant marrow with antileukemic reactivity to obtain a graft-versus-leukemia (GvL) effect against residual leukemic cells. Recently Weiden et al., (1979) presented evidence of a GvL effect in human recipients of marrow grafts from HLA compatible allogeneic donors. Unfortunately, this antileukemic effect was associated with moderate, severe or lethal graft versus host disease (GvHD), and the lower probability of recurrent leukemia was offset by a higher probability of GvHD related mortality. However, Bortin et al., (1979) demonstrated in mice that pool-alloimmunization in vivo generated cells which, when transplanted, killed disseminated AKRL cells in vivo. In this system a GvL reaction was evoked without augmentation of GvHD. Interestingly, Zarling et al., (1979) reported that T cells cytotoxic for autologous human leukemia cells could be generated in vitro by poolalloimmunization and that specific cytotoxic cells could be propogated indefinitely in vitro. Production of autologous tumor specific immunized lymphocytes in vitro and maintenance in long term cultures may thus permit clinical exploitation of the principles demonstrated in experimental models.

C. Macrophage Cytotoxicity:

Macrophages are large mononuclear phagocytic cells which are partially characterized by their tendency to adhere to glass or plastic surfaces and are found fixed in tissues, free in peritoneal exudates and in the circulation. Their participation is required for lymphocytes to produce antibody, cell-mediated responses and responses to mitogenic stimuli.

In addition to being an accessory cell in generation of cellular and humoral responses, macrophages can play a more direct role in the immune response by becoming "armed" or activated to be effector cells against parasites, bacterial cells, allogeneic cells and tumors. The mechanism of this cytotoxic effector function has long been of interest and recent work by Nathan and his colleagues at Rockefeller University is providing some new insight. In vitro studies have demonstrated that macrophages and granulocytes that have been pharmacologically triggered with phorbol myristrate produce large amounts of hydrogen peroxide. A comparison of eight types of effector cells including activated macrophages, demonstrated a correlation between extent of cytotoxicity to lymphoma cells and ability to generate peroxide. (Nathan et al., 1979 a). Further studies demonstrated that this release of hydrogen peroxide is necessary and sufficient for lymphoma cell lysis in this system (Nathan et al., 1979 b). Since both glucose and oxygen can be regarded as precursors to hydrogen peroxide generation, experiments depleting or depriving the in vitro system of either of these elements resulted in marked decrease or abolition of cytolysis of lymphoma cells by BCG activated macrophages.

Macrophages are a heterogeneous population and, like lymphocytes, this extends to functional as well as physical heterogeneity. Rabbit peritoneal exudate cells, rich in macrophages and separated into sub-populations on discontinuous density gradients, were analyzed for various functional capacities. These macrophages apparently separate according to degree of activation/maturation. A differing distribution between these populations in regard to lysozyme, and several lysomsomal acid hydrolases including acid phosphatase, has been demonstrated (Fishman et al., 1979). This and data for the other enzymes are interesting in light of the previously published studies showing that cells of particular size and density are effective in nonimmune-inhibition of syngeneic and xenogeneic tumor cell growth in vitro while cells of other densities actually are ineffective or promote growth of tumor cells. By working with purified sub-populations, it should be possible to resolve the biological significance of the various properties exhibited by macrophages and then apply that information in refined attempts at modulation of activity.

D. T Cell Suppressor Factors:

Recent studies in many laboratories have documented the concept that the many sub-populations of lymphocytes which exist are capable of interacting with another in a complex but highly regulated manner. As an example of this, one

can take some recent work on idiotypes. Antibodies which are capable of specifically recognizing the same or structurally related antigens themselves bear antigenic determinants called idiotypes. In a theory first published several years ago, Jerne postulated that idiotype-anti-idiotype interactions could provide a controlling mechanism for immune responses. Work by Benacerraf and his colleagues at Harvard exemplifies the fact that T (thymus-dependent) cells are involved in this mechanism. By the appropriate immunizations, it is possible to produce T cells which are capable of specifically suppressing the B cell response to a given antigen. These T cells contain a soluble factor capable of binding the same antigen (in this case, the azobenzenearsonate hapten) and this factor while not resembling immunoglobulin does bear the idiotypic determinant found on B cell immunoglobulin which reacts with the hapten (Greene, et al., 1979; Bach, et al., 1979). This is in accord with the concept that T cells may have receptors for antigen which resemble the V (variable) but not the C (constant) regions of immunoglobulin. Further, it indicates that T cells might also be regulated by an idiotypic network. Sy, et al., (1979 a,) took this one step further by showing that anti-idiotypic antibody could induce specific suppressor cells and could also reduce the level of a T cell delayed type hypersensitivity to the azobenzenearsonate hapten. The relevance of this to tumor immunology in practical terms is that attempts to strongly stimulate an anti-tumor response might be hampered by intrinsic regulatory forces on that response. By support of further basic work in this area, we may develop an understanding of how to bypass or override some regulator mechanisms and enhance anti-tumor responses.

By highlighting some recent work in these selected areas, it is evident that studies in tumor immunology have much to contribute to our understanding of the host-tumor relationship. Improved and new technologies, the use of tumors as model systems to explore basic problems in immunology and the feedback of this information upon applied clinical studies are opening doors where previously there were only walls. The investment in basic research within the Immunology Program over the past several years has resulted in a foundation that should be useful for future studies. Just as B cells, T cells and macrophages form a complex and highly interrelated network, so must our support be aimed at furthering the interrelationship of studies in a broad spectrum of areas.

Other Program Activities:

In order to encourage the rapid dissemination of research findings, the Immunology Program also supports meetings and conferences. Among those supported in whole or in part during this fiscal year are:

"The Eighth International Histocompatability Workshop and Conference" - Los Angeles, California - January 1979 - January 1980.

"Culture of Macrophage Workshop" - Lake Placid, New York - October 1979.

"Midwest Immunology Conference" - Detroit, Michigan - November 1979.

"International Symposium on Immunological Memory" - Tampa, Florida - December 1979.

"Conference on the Biology of Bone Marrow" - Keystone, Colorado - February 1980.

"Gordon Conference on Immunochemistry" - Santa Barbara, California - February 1980.

"Symposium Liposomes and Immunobiology" Houston, Texas - March 1980.

"The Second International Meeting on Immunotherapy of Cancer - Present Status of Trials in Man" - Bethesda, Maryland - April 1980.

"Immune Responsiveness and Experimental Models of Infections and Oncologic Diseases" - Dallas, Texas - June 1980.

"The Eighth International Congress of the Transplantation Society" - Boston, Massachusetts - June-July 1980.

"International Symposium on the Ruminant Immune System" - Plymouth, New Hampshire - July 1980.

"4th International Congress on Immunology" - Paris, France - July 1980.

"An International Workshop on Monuclear Phagocytes" - Vienna, Austria - July 1980.

"Cell Surface Receptors in Immune Mechanisms" - Warsaw, Poland - July 1980.

"Immune Intervention - 1980's" - Santa Fe, New Mexico - October 1980.

"The Continous Culture of Functional T-Cells" - Holderness, New Hampshire - October 1980.

"The Second International Conference on B Lymphocytes in the Immune Response" Scottsdale, Arizona - October 1980.

REFERENCES

Ault, K.A., "Detection of Small Numbers of Monoclonal B Lymphocytes in the Blood of Patients with Lymphoma," New Engl. J. Med., 300:1401-1405, 1979. (CA 14723)

Bach, B.A., Greene, M.J., Benacerraf, B. and Nisonoff, A., "Mechanisms of Regulation of Cell Mediated Immunity. IV. Azobenzenearsonate Specific Suppressor Factors Bear Cross Reactive Idiotypic Determinants the Expression of Which Is Linked to the Heavy Chain Altotype Linkage Group of Genes," J. Exp. Med. 149:1084-1098, 1979. (CA 14723)

Bortin, M.M., Truitt, R.L., Rimm, A.A. and Bach, F.H., "Graft-versus-Leukemia Reactivity Induced by Alloimmunization without Augmentation of Graft-versus-Host Reactivity," Nature, 281:490-491, 1979. (CA 20484 and CA 18440)

Cancro, M.P., Wylie, D.E., Gerhard, W., and Klinman, N.R., "Patterned Acquisition of the Antibody Repertoire: Diversity of the Hemagglutinin-Specific B-Cell Repertoire in Neonated Balb/c Mice," Proc. Nat'l. Acad. Sci. (U.S.) 76:6577, 1979. (CA 15822)

Celis, E., Chang, T.W., and Eisen, H.N., "Cyclical Changes in Susceptibility of a Myeloma Tumor (LPC-1) to Immune Destruction III (Periodic Production of a Cell Surface Glycoprotein and Changes in Reactivity with Cytotoxic T Cells and Anti-H-2 Sera," J. Immunol., 122:2245-2250, 1979. (CA 15472)

Dupont, B., O'Reilly, R.J., Pollack, M.S., and Good, R.A., "Use of HLA Genotypically Different Donors in Bone Marrow Transplantation," Transplant. Proc., 11:219-224, 1979. (CA 22507)

Fefer, A., Cheever, M.A., Thomas, E.D., Boyd, C., Ramberg, R., Glucksberg, H., Buckner, C.D. and Storb, R., "Twin-Marrow Transplantation for Chronic Granulocyte Leukemia," New Engl. J. Med., 300:333-337, 1979. (CA 18221)

Fishman, M., and Weinberg, D.S., "Functional Heterogeneity Among Peritoneal Macrophages, II, Enzyme Content of the Macrophage Subpopulations," Cell. Immunol., 45:437-445, 1979. (CA 18672)

Greene, M.J., Bach, B.A., and Benacerraf, B., "Mechanisms of Regulation of Cell Mediated Immunity: III. The Characterization of Azobenzenearsonate - Specific Suppressor T Cell Derived Suppressor Factors," J. Exp. Med., 149:1069-1083, 1979. (CA 14723)

Hale, A.H., Celis, E., Russell, J.H., and Eisen, H.N., "Cyclical Changes in Susceptibility of a Myeloma Tumor (LPC-1) to Immune Destruction II. Periodic Fluctuations During Growth in Normal and Nude Mice and in Culture," J. Immunol., 122:959-964, 1979. (CA 15472)

Herlyn, M., Steplewski, Z., Herlyn, D., and Koprowski, H., "Colorectal Carcinoma-Specific Antigen: Detection by Means of Monoclonal Antibodies," Proc. Natl. Acad. Sci. (U.S.) 76:1438-1442, 1979. (CA 25874)

Knapp, M.R., Severinson-Gronowicz, E., Schroder, J., and Strober, S., "Characterization of a Spontaneous Murine B Cell Leukemia II. Tumor Cell Proliferation and IgM Secretion after Stimulation by LPS," J. Immunol., 123:1000-1006, 1979. (CA 27942)

Krolick, K.A., Isakson, P.C., Uhr, J.W., and Vitetta, E.S., "Murine B Cell Leukemia (BCL-1): Organ Distribution and Kinetics of Growth as Determined by Fluorescence Analysis with an Anti-Idiotypic Antibody," J. Immunol., 123:1928-1935, 1979. (CA 23115)

Kubagawa, H., Vogler, L.B., Capra, J.D., Conrad, M.E., Lawton, A.R., and Cooper, M.D., "Studies on the Clonal Origin of Multiple Myeloma. Use of Individually Specific (Idiotype) Antibodies to Trace the Oncogenic Event to its Earliest Point of Expression in B-Cell Differentiation," J. Exp. Med., 150:792-807, 1979. (CA 16673)

Le Bien, T.W., Hozier, J., Minowada, J., and Kersey, J.H., "Origin of Chronic Myelocytic Leukemia in a Precursor of Pre-B Lymphocytes," New Engl. J. Med. 301:144-147, 1979. (CB 84261 and CA 25097)

Ligler, F.S., Vitetta, E.S., Graham Smith, R., Himes, J.B., Uhr, J.W., Frenkel, E.P., and Kettman, J.R., "An Immunologic Approach for the Detection of Tumor Cells in the Peripheral Blood of Patients with Malignant Lymphoma; Implications for the Diagnosis of Minimal Disease," J. Immunol., 123:1123-1126, 1979. (CA 23115)

Nathan, C.F., Brukner, L.H., Silverstein, S.C., and Cohn, Z.A., "Extracellular Cytolysis by Activated Macrophages and Granulocytes, I. Pharmacologic Triggering of Effector Cells and the Release of Hydrogen Peroxide," J. Exp. Med., 149:84-99, 1979 a. (CA 22090)

Nathan, C.F., Silverstein, S.C., Brukner, L.H., and Cohn, Z.H., "Extracellular Cytolysis by Activated Macrophages and Granulocytes, II Hydrogen Peroxide as a Mediator of Cytotoxicity," J. Exp. Med., 149:100-113, 1979 b. (CA 22090)

Reinberg, E.L., Kung, P.C., Goldstein, G. and Schlossman, S.F., "A Monoclonal Antibody with Selective Reactivity With Functionally Mature Human Thymocytes and All Peripheral Human T Cells," J. Immunol., 123:1312-1317, 1979. (CA 25369)

Steplewski, Z., Herlyn, M., Herlyn, D., Clark, W.H., and Koprowski, H., "Reactivity of Monoclonal Anti-Melanoma Antibodies with Melanoma Cells Freshly Isolated from Primary and Metastatic Melanoma," Eur. J. Immunol., 9:94-96, 1979. (CA 25874)

Sy, M.S., Bach, B.A., Dahi, Y., Nisonoff, A., Benacerraf, B. and Greene, M.J. "Antigen and Receptor Driven Regulatory Mechanisms. I. Induction of Suppressor Cells with Anti-Idiotypic Antibodies," J. Exp. Med., 150:1216-1228, 1979. (CA 14723)

Thomas, E.D., Sanders, J.E., Flournoy, N. Johnson, F.L., Buckner, C.D., Clift, R.A., Fefer, A., Goodell, B.W., Storb, R. and Weiden, P.L., "Marrow Transplantation for Patients with Acute Lymphoblastic Leukemia in Remission," Blood, 54:468-476, 1979. a. (CA 18029)

Thomas, E.D., Buckner, C.D., Clift, R.A., Fefer, A., Johnson, F.L., Neiman, P.E., Sale, G.E., Sanders, J.E., Singer, J.W., Shulman, H., Storb, R., and Weiden P.L., "Marrow Transplantation for Acute Nonlymphoblastic Leukemia in First Remission," New Engl. J. Med., 301:597-599, 1979. b. (CA 18029)

Warner, N.L., Daley, M.J., Richey, J. and Spellman, C., "Flow Cytometry Analysis of Murine B Cell Lymphoma Differentiation," Immunol. Rev., 197-243, 1979. (CA 22105)

Weiden, P.L., Flournoy, N., Thomas, E.D., Prentice, R., Fefer, A., Buckner, C.D., and Storb, R., "Anti-leukemic Effect of Graft-versus-Host Disease in Marrow Transplantation," New Engl. J. Med., 300:1968, 1973, 1979. (CA 18221 and (CA 18029)

Zarling, J.M. and Bach, F.H., "Continuous Culture of T Cells Cytotoxic for Autologous Human Leukemic Cells," Nature, 280:685-687, 1979. (CA 27477)

Yeh, M.Y., Hellstrom, I., Brown, J.P., Warner, G.A., Hansen, J.A., and Hellstrom, K.E., "Cell Surface Antigens of Human Melanoma Identified by Monoclonal Antibody," Proc. Natl. Acad. Sci. (U.S.) 76:2927-2931, 1979. (CA 19149)

ANTIBODY STRUCTURE, SYNTHESIS, AND FUNCTION

R01-CA-21923 Oligosaccharide Structure and Receptor Specificity
Jacques U. Baenziger Washington University

R01-CA-25958 Organ Specific Tumor Localizing Antibody
William F. Bale Georgia Institute of Technology

P01-CA-25319 Synthesis of Human Myeloma Variable Domains in E. coli
Sherman Beychok Columbia University

R01-CA-13014 Proteins of Plasma Cell Cancers
Sherman Beychok Columbia University

R01-CA-23967 Radiolabeled Antibody Tumor Localization
Donald J. Buchsbaum University of Minnesota

R01-CA-25049 Structure and Genetics of Antibody Variable Regions
Lovick E. Cannon Brandeis University

R23-CA-28046 The Immunoregulatory Role of IgD
Marvin A. Cuchens University of Mississippi Medical Center

R01-CA-22126 Ultraviolet Light Radiation and Immunoregulation
Raymond A. Daynes University of Utah

R01-CA-19616 Immunoglobulins in Multiple Myeloma and Amyloidosis
Allen B. Edmundson University of Utah

R01-CA-15215 Structural Studies of Unique Myeloma Immunoglobulins
Frederick A. Garver Medical College of Georgia

R01-CA-24432 Sequence, Shape and Specificity of Antibodies
Edgar Haber Massachusetts General Hospital

R01-CA-25044 Surface IgM of Malignant Lymphocytes and Plasma Cells
Scot G. Hickman Jewish Hospital of St. Louis

R01-CA-23404 Serologic Screening for Contagion of Human Sarcomas
Yashar Hirshaut Sloan Kettering Institute for Cancer Research

R01-CA-20818 Variable Regions of Biclonal Immunoglobulins
Robert G. Krueger Christ Hospital

R01-CA-26440 Tumor Cells Escape from Immune Effector Mechanisms
Chao-Ying Kuo Barbara Kopp Geriatric Research Center

P01-CA-16835 Studies of Monoclonal Gammopathies in Humans
Robert A. Kyle Mayo Foundation

R01-CA-23885 Secretory Immunoglobulin
Michael E. Lamm New York University

R01-CA-17531 Mechanism and Uses of Anti-Ig Immunosuppression
Dean D. Manning University of Wisconsin

R01-CA-22491 Characterization of Precursor Proteins and Fc Receptor
David J. McKean Mayo Foundation

R01-CA-24484 Monitoring Ig-Secreting Cells
Giuseppe A. Molinaro Scripps Clinic and Research Foundation

R01-CA-16858 Genetics and Biochemistry of Myeloma Ig Production
Sherie L. Morrison Columbia University

R01-CA-20045 Antibody Mediated Cell-Cell Interactions
Julia M. Phillips-Quagliata New York University

R01-CA-08497 Abnormal Proteins in Multiple Myeloma
Frank W. Putnam Indiana University

R01-CA-25758 Biochemical Studies on Membrane Bound Immunoglobulin
Frank F. Richards Yale University

R01-CA-23028 Molecular Studies of the Immune Response
John H. Richards California Institute of Technology

R01-CA-10267 Immunological Lysis in Neoplastic Disease
Wendell F. Rosse Duke University

R01-CA-25048 X-Ray Crystal Structure and Function of Proteins
Raghupathy Sarma State University of New York

R01-CA-20075 Antibody Affinity in Immune Response and Tolerance
Gregory W. Siskind Cornell University Medical Center

R01-CA-10056 Proteins in Multiple Myeloma & Related Blood Diseases
Alan Solomon University of Tennessee

R01-CA-25754 Control of Immunoglobulin Synthesis
Ursula B. Storb University of Washington

R01-CA-25166 Defects in Immunoglobulin Production in Mouse Myeloma
Stephen Weitzman State University of New York

R01-CA-20088 Tumor-Bound Ig--An Expression of Antitumor Immunity
Isaac P. Witz Tel Aviv University

R01-CA-22613 Humoral Cytotoxic Response in Glioma Patients
William C. Wood Massachusetts General Hospital

IDENTIFICATION, ISOLATION, AND CHARACTERIZATION OF CELL SURFACE ANTIGENS

R01-CA-15338 Membrane Specificities Related to Neoplasia
Ronald T. Acton University of Alabama

R01-CA-26321 Cell-Surface Antigens of Murine Tumors
James P. Allison University of Texas System Cancer Center

R01-CA-26891 Surface Antigens of Rat Hepatocellular Carcinomas
James P. Allison University of Texas System Cancer Center

R01-CA-12389 Immunochemical Studies of Gastrointestinal Cancer
Elliot E. Alpert Massachusetts General Hospital

R01-CA-21651 Teratocarcinoma and Embryonal Tumors: Surface Antigens
Karen Artzt Sloan Kettering Institute for Cancer Research

R01-CA-15556 Antigens and Immunity in Human Lung Cancer
C. Elliott Bell, Jr. Washington University

R01-CA-22790 Cell Surface Antigens Glial Brain Tumor Cells
Darell D. Bigner Duke University

R01-CA-24358 Leukemia Associated Antigens
Ronald J. Billing University of California, Los Angeles

R01-CA-24314 Inappropriate Antigens on SJL/J Reticulum Cell Sarcoma
Benjamin Bonavida University of California, Los Angeles

R01-CA-27841 Antigens of Chemically Transformed Mouse Fibroblasts
Joseph P. Brown Fred Hutchinson Cancer Research Center

R01-CA-27534 Nucleolar Antigens of Human Cancer Cells
Harris Busch Baylor College of Medicine

R01-CA-13844 Isolation of Tumor Antigens of Human Melanoma
Jean-Claude Bystryn New York University

R01-CA-25171 Cell Surface Antigens of Murine Tumors
Gerald N. Callahan Scripps Clinic and Research Foundation

R01-CA-26456 Cell Surface Antigens in Murine Leukemia
Gerald N. Callahan Scripps Clinic and Research Foundation

R01-CA-28564 Human Squamous Cell Carcinoma: Culture and Serology
Thomas E. Carey University of Michigan

R01-CA-26226 Childhood Neuroblastoma: Antigenic Characteristics
James T. Casper Medical College of Wisconsin

R23-CA-27893 Bence Jones Protein Properties & Tubulotoxicity
Praveen N. Chander New York Medical College

R01-CA-03842 Experimental Bladder Tumors
Warren H. Chapman University of Washington

R01-CA-15064 Immunochemical Studies on Carcinogenic Mycotoxins
Fun S. Chu University of Wisconsin

R01-CA-22674 Characterization of Fetal Antigens in Tumors
Joseph H. Coggin, Jr. University of South Alabama

R01-CA-21279 Isolation and Characterization of Fc Receptors
Sheldon M. Cooper University of Southern California

R01-CA-20500 Structural and Serological Studies on Ia Antigens
Susan E. Cullen Washington University

R01-CA-27063 Genetics of Cytotoxicity to Virus Modification in Man
Devendra P. Dubey Sidney Farber Cancer Institute

R01-CA-14346 Human Tumor-Associated Molecular Expressions
Thomas S. Edgington Scripps Clinic and Research Foundation

R01-CA-24437 Expression of T Lymphocyte Differentiation Antigens
Walter J. Esselman Michigan State University

R01-CA-16069 Immunochemical Characterization of Antigens in Melanoma
Soldano Ferrone Scripps Clinic and Research Foundation

R01-CA-24329 Monoclonal Antibodies to Human Cell Surface Antigens
Soldano Ferrone Scripps Clinic and Research Foundation

R01-CA-28746 Cell Surface Antigens in Neoplastic & Autoimmune Disease
Robert I. Fox Scripps Clinic and Research Foundation

R01-CA-20820 Structural Studies of the Products of the H-2 Complex
John H. Freed Johns Hopkins University

P01-CA-23115 New Immunologic Approaches to Lymphoid Neoplasms
Eugene P. Frenkel University of Texas Health Sciences Center

R01-CA-26479 Immune Functions of Tumor Cell Variants
Hiroshi Fuji Roswell Park Memorial Institute

R01-CA-18892 MLR Locus Typing of Cancer Patients
Richard A. Gatti Cedars-Sinai Medical Center

R01-CA-14061 Chicken Lymphocyte Alloantigens and Viral Oncogenesis
Douglas G. Gilmour New York University

R01-CA-18641 Brain Associated Hematopoietic Antigens
Edward S. Golub Purdue University

R01-CA-26476 Membrane Antigen Organization in Tumor Immunity
Linda R. Gooding Duke University

R01-CA-19224 Relation of Blood Group and Human Tumor Antigen
Sen-Itiroh Hakomori Fred Hutchinson Cancer Research Center

R01-CA-25508 Ontogenetic Relationship of CR Plus/CA-B Lymphocytes
Ulrich G. Hammerling Sloan Kettering Institute for Cancer Research

R01-CA-25558 Cell Surface Antigens of Chemically Induced Sarcomas
Karl E. Hellstrom Fred Hutchinson Cancer Research Center

R01-CA-28619 Purification of Human Sarcoma Heterophile Antigens
Yashar Hirshaut Sloan Kettering Institute for Cancer Research

R01-CA-28230 Swine Melanoma Antigens: Isolation and Evaluation
Reuel R. Hook, Jr. University of Missouri

R01-CA-25873 Membrane Proteins of Human Leukemias and Lymphomas
Robert E. Humphreys University of Massachusetts Medical School

R01-CA-22093 Functions of Atypical Lymphocytes
Lindsey M. Hutt-Fletcher University of North Carolina

R01-CA-25893 Cell Surface Molecules: Hematopoietic Differentiation
Robert A. Hyman Salk Institute for Biological Studies

R01-CA-18734 Immunologic Studies Related to Malignancy
Carol A. Jones University of Colorado Medical Center

R01-CA-27124 Molecular Approaches to Human Colon Cancer
Barry D. Kahan University of Texas Health Sciences Center

R01-CA-17534 Lymphocyte Differentiation in Childhood Leukemia
Joseph Kaplan Children's Hospital of Michigan

R01-CA-25097 Differentiation of Immune System: Cell Surface Antigens
John H. Kersey University of Minnesota

R01-CA-18470 Antigenicity and Tumorigenicity of Somatic Cell Hybrids
Barbara B. Knowles Wistar Institute of Anatomy and Biology

P01-CA-25874 Human Melanoma and Tumor Specific Monoclonal Antibodies
Hilary Koprowski Wistar Institute of Anatomy and Biology

R01-CA-15318 Neutrophil Antigens; Pathophysiology & Chemistry
Parviz Lalezari Montefiore Hospital and Medical Center

R23-CA-28526 Analysis of Human Leukemia Cells with Hybridomas
Tucker W. Le Bien University of Minnesota

R01-CA-22541 Purification of Human Breast Tumor Proteins
Michael P. Lerner University of Oklahoma Health Sciences Center

R01-CA-25910 Production of Antibodies to Tumor Associated Antigens
Kiu Leung Rutgers, The State University

R01-CA-28448 Forssman Antigen/Antibody and Human Adenocarcinoma
Philip Levine Sloan Kettering Institute for Cancer Research

R01-CA-20660 Mutant A, P1 Antigens in Malignancy, Cytotoxicity
Philip Levine Sloan Kettering Institute for Cancer Research

R01-CA-21223 Antitumor Antibodies Generated In Vitro
Ronald Levy Stanford University

R01-CA-20168 Neoplastic and Normal Cell Thymus-Leukemia Antigens
J. Russell Little, Jr. Jewish Hospital of St. Louis

R01-CA-26184 Antigens of Human Ovarian Tumors
Kenneth O. Lloyd Sloan Kettering Institute for Cancer Research

R01-CA-17192 Study of Human Multiple Myeloma Cell Antigens
Malcolm R. MacKenzie University of California, Davis

R01-CA-07973 Role of Transplantation Antigens in Malignant Cells
Lionel A. Manson Wistar Institute of Anatomy and Biology

R01-CA-21431 Role of Circulating Antigen in Tumor Immunity
Charles F. McKhann University of Minnesota

R01-CA-25852 Isolation and Characterization of Heterophile Antigens
Joseph M. Merrick State University of New York

R01-CA-08975 Human Leukemia Associated Antigens
Richard S. Metzgar Duke University

R01-CA-15494 Tolerance & Immunity to Avian RNA Tumor Viruses & Visa
Paul Meyers Mayo Foundation

R01-CA-27628 Studies on Tumor-Specific and Tumor-Associated Antigens
Felix Milgrom State University of New York

R01-CA-27416 Characterization of New Human Ia and Leukemia Antigen
Thalachallour Mohanakumar Virginia Commonwealth University

R01-CA-25052 Immune Responses In Vitro-H-2 (Ir) Locus Function
John E. Niederhuber University of Michigan

P01-CA-19765 Human Cancer Serology
Lloyd J. Old Sloan Kettering Institute for Cancer Research

R01-CA-27168 Histamine H2 Receptor T-Suppressor Cells in Cancer
Michael E. Osband University Hospital

R01-CA-24910 Oncofetal Antigens in Embryogenesis and Tumor Growth
Suzanne Ostrand-Rosenberg University of Maryland

R01-CA-21912 Antigens Induced by Polyoma Virus Transformation
Samuel J. Pancake Georgetown University

R01-CA-16071 Urine as Source of Human Cell Surface Markers
Michele A. Pellegrino Scripps Clinic and Research Foundation

R01-CA-27134 Immunobiology of Human Malignant Melanoma
Jerry W. Pickering Louisiana State University Medical Center

R01-CA-25134 Lymphocyte Surface Glycoconjugates
Ronald D. Poretz Rutgers, The State University

R01-CA-17276 Membrane Antigens from Normal and Leukemic Lymphocytes
David Pressman Roswell Park Memorial Institute

R01-CA-28420 Molecular Profile of Human Melanoma Antigens
Ralph A. Reisfeld Scripps Clinic and Research Foundation

R01-CA-19108 Neoplasia and Histocompatibility Antigens in Serum
Ralph A. Reisfeld Scripps Clinic and Research Foundation

R01-CA-28253 Immune Response to Syngeneic Leukemic B Cell Antigens
Manuel J. Ricardo, Jr. University of Tennessee Center Health Sciences

R01-CA-24726 Immune Mechanisms Involved in Tumor Metastasis
Oliver A. Roholt, Jr. Roswell Park Memorial Institute

P01-CA-18900 Program in Cancer Immunology
Noel R. Rose Wayne State University

R01-CA-28416 Receptors for Immunoglobulin on Human Lymphoma-Leukemia
Richard A. Rudders New England Medical Center Hospital

R01-CA-27460 Alpha-Fetoprotein: Structure and Function
Erkki I. Ruoslahti La Jolla Cancer Research Foundation

R01-CA-12851 Embryonic & Virally Induced Tumor-Cell Membrane Antigens
Bob G. Sanders University of Texas

R01-CA-25369 Human Leukemia Antigens: Isolation & Characterization
Stuart F. Schlossman Sidney Farber Cancer Institute

R01-CA-24433 Structures of Histocompatibility-2 Membrane Antigens
Duane W. Sears University of California, Santa Barbara

R01-CA-22794 Human Neuroblastoma Cell Surface Antigens
Robert C. Seeger University of California, Los Angeles

R01-CA-19304 Human Leukemia and Lymphoma Associated Antigens
Ben K. Seon Roswell Park Memorial Institute

R01-CA-27471 Modulation of Cellular Responses by Membrane Fluidity
Meir Shinitzky Weizmann Institute of Science

R01-CA-22211 Tumor Rejection Antigens from Treated Cancer Cells
Selma Silagi Cornell University Medical Center

R01-CA-23030 Structure Studies of Ia Alloantigens
Jack Silver Scripps Clinic and Research Foundation

R01-CA-22360 Fetal Isoenzyme Expression in Human Tumor Xenografts
Robert M. Singer Fairleigh Dickinson University

R01-CA-22034 Differentiation Antigens of Human Lymphoblastoid Cells
R. Graham Smith University of Texas Health Sciences Center

R01-CA-25045 Neutrophil Antigens in Immune Neutropenia
Wade K. Smith Virginia Commonwealth University

R01-CA-25062 Secretory Component in Neoplasia
Mary A. South Texas Tech University

R01-CA-22540 Human Cancer Relation of MN and Precursor Structures
Georg F. Springer Evanston Hospital

R01-CA-27547 Chemistry of Tumoricidal Macrophage Surface Antigens
Timothy A. Springer Harvard University

R01-CA-22128 Antisera to Human Leukemia-Associated Antigens
Robert N. Taub Virginia Commonwealth University

R01-CA-23322 Circulating and Cell Bound Immune Complexes
Argyrios N. Theofilopoulos Scripps Clinic and Research Foundation

R01-CA-18946 Cell Surface Modulation in Mammary Neoplasia
Karen E. Thompson Children's Hospital Medical Center Northern California

R01-CA-22964 Cross-Reactions Between Fetal, Sperm and Tumor Cells
Sei Tokuda University of New Mexico

R01-CA-24024 In Vitro Generation of Immunity to Human Colon Cancer
Baldwin H. Tom University of Texas Health Sciences Center

R01-CA-20272 Identification of Human Lymphocyte Surface Antigens
Ian S. Trowbridge Salk Institute for Biological Studies

R01-CA-22902 Serodetection of Neoantigens in Urogenital Malignancies
Karen S. Webb Duke University

R23-CA-26584 The Molecular Nature of Tumor Antigens
Richard G. Woodbury Fred Hutchinson Cancer Research Center

R01-CA-27789 Human Antibodies to Melanoma
Peter W. Wright Fred Hutchinson Cancer Research Center

R23-CA-27746 Role of Glycolipids in Immune Cell Functions
William W. Young, Jr. Fred Hutchinson Cancer Research Center

CELLULAR IMMUNOLOGY

R01-CA-16784 Tumoricidal Effects of Macrophages: Pathologic Study
Dolph O. Adams Duke University

R01-CA-25036 Studies of Lymphocyte-Defined HLA and B-Cell Antigens
Richard J. Albertini University of Vermont & St. Agric. College

R01-CA-23886 Role of Hapten-Bearing Cell in Tolerance
Marlene A. Aldo-Benson Indiana University-Purdue University

R01-CA-14049 Cell-Mediated Immunity to Ascites Tumors
D. Bernard Amos Duke University

R01-CA-24067 Fc Receptor Structure and Function
Clark L. Anderson University of Rochester

R01-CA-24728 Tumor Induced Changes in Lymphatic Tissues
Norman D. Anderson Johns Hopkins University

R01-CA-15462 Cell Interactions in Tumor Immunity
Bertie F. Argyris Upstate Medical Center

R01-CA-27826 Manipulation of Antitumor Immunity In Vitro
Fritz H. Bach University of Minnesota

R01-CA-22786 Receptor Dynamics and Normal/Tumor Cell Function
Richard B. Bankert Roswell Park Memorial Institute

R01-CA-25253 Immunoregulatory Network Probed by Cell Hybridization
Richard B. Bankert Roswell Park Memorial Institute

R01-CA-24472 Development of Thymic Lymphocytes
Ross S. Basch New York University

R23-CA-28368 Thymus Cell Maturation and Leukemogenesis in Mice
Terry R. Beardsley University of California, San Diego

R01-CA-27854 Cell Surface Carbohydrate and Lymphocyte Interactions
John E. Bell University of Rochester

P01-CA-14723 Study of Experimental Cancer Immunology
Baruj Benacerraf Harvard University

R01-CA-24431 Cellular and Structural Basis of Immunological Tolerance
David C. Benjamin University of Virginia

R01-CA-25053 Selective Stimulation of Cell Mediated Immunity
Eliezer Benjamini University of California, Davis

R01-CA-19753 Mixed Leukocyte Tumor Reaction in Syngeneic Systems
Benjamin Bonavida University of California, Los Angeles

R01-CA-22957 Neoplasm and Resistance to Infections
Peter F. Bonventre University of Cincinnati

R01-CA-20093 Comparative Immunobiology
Myrin Borysenko Tufts University

R01-CA-18602 Immunocompetent Cells in Acute Lymphocytic Leukemia
James T. Casper Medical College of Wisconsin

R01-CA-28504 T Cell Growth and Differentiation in Leukemia
Jen W. Chiao Sloan Kettering Institute for Cancer Research

P01-CA-17404 Immunobiology, Immunodeficiency, and Cancer
Yong S. Choi Sloan Kettering Institute for Cancer Research

R01-CA-24353 Neutrophil-Mediated Tumor Cell Cytotoxicity
Robert A. Clark University Hospital

R01-CA-21329 Recruitment of T Cell Immunity to Antigens and Tumors
Irun R. Cohen Weizmann Institute of Science

R01-CA-20822 Cell Interaction and the Clotting System
Robert B. Colvin Massachusetts General Hospital

P01-CA-16673 Cell Differentiation Studies in Cancer Immunobiology
Max D. Cooper University of Alabama

R01-CA-14692 Cancer Cell Survival and Mechanisms of Cytotoxicity
Neil R. Cooper Scripps Clinic and Research Foundation

R01-CA-22212 Scanning EM for Lymphocytes with Markers for Surface Ig
Etienne De Harven Sloan Kettering Institute for Cancer Research

R01-CA-19334 Antigen Receptor of Continuous T Killer Cell Line
Gunther Dennert Salk Institute for Biological Studies

R01-CA-15581 Immune Response to Tumor Cell Surface Antigens
Gunther Dennert Salk Institute for Biological Studies

R01-CA-25391 Immunological Control of Dormant Leukemia
Michael I. Dietz Michigan Cancer Foundation

R01-CA-25043 Regulation of Immunity at the Molecular Level
Howard M. Dintzis Johns Hopkins University

R01-CA-21900 Protective T-Cell Subpopulation Responses in Leukemia
Paul S. Duffey University of Texas Health Sciences Center

R01-CA-28834 Basophil/Mast Cell Function in the Control of Cancer
Ann M. Dvorak Beth Israel Hospital

R01-CA-28471 Biology of Solid Tumor Growth and Immune Rejection
Harold F. Dvorak Beth Israel Hospital

R01-CA-23503 Mechanisms of Macrophage Antitumor Activation
Paul J. Edelson Children's Hospital Medical Center

R01-CA-20499 Immunobiology of Cutaneous T Cell Lymphomas
Richard L. Edelson Columbia University

R01-CA-23679 Cell Mediated Hyperacute Rejection
Ernst J. Eichwald University of Utah

R01-CA-15472 Immunity to Myeloma Tumors
Herman N. Eisen Massachusetts Institute of Technology

R01-CA-16271 Regulatory Signals in Cytotoxic T-Cell Development
Ronald B. Faanes Sloan Kettering Institute for Cancer Research

P01-CA-12800 Immune Functions and Cancer
John L. Fahey University of California, Los Angeles

R01-CA-27915 Antibody Dependent Cell Cytotoxicity Reactions
Michael W. Fanger Case Western Reserve University

R01-CA-25756 Regulation of Cell Growth by 5'-Methylthioadenosine
Adolph J. Ferro Oregon State University

R01-CA-18672 The Role of Macrophage Subclasses in Tumor Immunity
Marvin Fishman St. Jude Children's Research Hospital

R01-CA-23477 Immunologic Inhibition of Tumor Growth
James T. Forbes Vanderbilt University

R01-CA-25411 SJL Mice Lymphomagenesis as a Model of B-Cell Lymphoma
Richard J. Ford, Jr. University of Texas System Cancer Center

R01-CA-24196 Ultraviolet Carcinogenesis and Immunity
G. William Fortner Kansas State University

R23-CA-27542 T Cell Subsets and Marek's Disease Viral Oncogenesis
Tommy L. Fredericksen New York University

R01-CA-24338 In Vitro Studies of Normal and Neoplastic Lymphocytes
Shu Man Fu Rockefeller University

R01-CA-14216 Characterization of Lymphoid Populations in Cancer
Richard K. Gershon Yale University

R01-CA-28419 Control of Normal and Leukemic T-Cell Proliferation
Steven Gillis Fred Hutchinson Cancer Research Center

R01-CA-25747 Histocompatibility Antigens in Defined Membranes
Penny J. Gilmer Florida State University

R01-CA-20169 Long-Lived Lymphocytes and Secretory Bursal Cells
Bruce Glick Mississippi State University

R23-CA-27552 Cytotoxic T Cells to Syngeneic MuLV+ Tumors
William R. Green Fred Hutchinson Cancer Research Center

P01-CA-21825 Self-Nonself Discrimination and Tumor Recognition
Howard M. Grey National Jewish Hospital & Research Center

R01-CA-20178 Macrophage Proliferation and Activation
John W. Hadden Sloan Kettering Institute for Cancer Research

R01-CA-24444 Regulation of Lymphocyte Activity by Proteases
David A. Hart University of Texas Health Sciences Center

R01-CA-19149 Transplantation Antigenicity of Virus Induced Tumors
Ingegerd E. Hellstrom Fred Hutchinson Cancer Research Center

R01-CA-24537 Murine Effector Cells
Christopher S. Henney Fred Hutchinson Cancer Research Center

R01-CA-20078 Mode of Action of a Stimulator of Tumor Resistance
Diane M. Jacobs State University of New York

R01-CA-25040 Differentiation of Human Mononuclear Phagocytes
Warren D. Johnson Cornell University Medical Center

R01-CA-25042 Immunology of Thy-1-Bearing Lymphocyte Subsets
Felipe Kierszenbaum Michigan State University

R01-CA-24215 Mechanisms of Metastasis
Untae Kim Roswell Park Memorial Institute

R01-CA-25384 Natural and Antibody-Dependent Cellular Cytotoxicity
Yoon B. Kim Sloan Kettering Institute for Cancer Research

R01-CA-25250 Natural Killer Cells: Genetic Control and Role
Eva Klein Caroline Institute

R01-CA-23354 Natural Tumor Cell Killing in Humans
Hillel S. Koren Duke University

R01-CA-22869 Significance of Tumor Leucocytic Infiltrates
John W. Kreider Pennsylvania State University

R01-CA-21401 Studies of Isolated Marrow-Dependent M Cells
Vinay Kumar Boston University

R01-CA-17273 The Immune Response to Virally Determined Tumor Antigens
Eddie W. Lamon University of Alabama

R01-CA-24335 Lectin-Dependent Cell-Mediated Cytotoxicity
David C. Laux University of Rhode Island

P01-CA-16247 Immunologic Resistance to Cancer
H. Sherwood Lawrence New York University

R23-CA-25668 Induction of Genetically Tailored Immune Imbalance
Stephen H. Leech Louisiana State University Medical Center

R01-CA-20812 Biological Activities of Homogeneous Mitogens
Myron A. Leon Wayne State University

R01-CA-23708 Regulator Lymphocytes of Dysgammaglobulinemic Chicks
Gerrie A. Leslie University of Oregon Health Sciences Center

R01-CA-13070 Immunity to Human Cancer--Functional Components
Nelson L. Levy Duke University

R01-CA-25583 Cell Mediated Immunity in Mouse Mammary Tumor Models
Diana M. Lopez University of Miami

R01-CA-28332 In Situ Anti-Tumor Immunity and Effects of Radiation
Edith M. Lord University of Rochester

R01-CA-23217 Immunologic Regulation of Myeloma Cell Growth
Richard G. Lynch Washington University

R01-CA-22492 Regulation of B-Lymphocyte Colonies
Donald Metcalf Walter and Eliza Hall Institute Medical Research

R01-CA-13396 Immunogenesis from Bone Marrow Cells
Harold C. Miller Michigan State University

R01-CA-15446 Antigen-Antibody Interactions in Neoplastic Disease
Percy Minden National Jewish Hospital & Research Center

R01-CA-25056 Immunoregulatory Effects of Bacterial Substances
Robert I. Mishell University of California, Berkeley

R01-CA-19052 Development and Function of Activated Macrophages
Malcolm A. Moore Sloan Kettering Institute for Cancer Research

R01-CA-24686 Macrophage Extrinsic Activity vs Viruses
Page S. Morahan Virginia Commonwealth University

R01-CA-25054 Cellular Mechanisms Regulating Antibody Production
Helen B. Mullen University of Missouri

R01-CA-13701 Mechanisms of Immunity in Leukemia
William H. Murphy University of Michigan

R01-CA-23874 Immune Response to Moloney Induced YAC Tumor
David Naor Hebrew University of Jerusalem

R01-CA-22090 Antitumor Action of Phagocytes and Lymphocytes
Carl F. Nathan Rockefeller University

R01-CA-22090 Mononuclear Leukocytes in Tumor Immunity
Carl F. Nathan Rockefeller University

R01-CA-12779 Leukocyte Regulatory Mechanisms
Peter C. Nowell University of Pennsylvania

R23-CA-28433 Identification of Ag Specific Suppressor Cells in Man
Richard C. Ostenson Fred Hutchinson Cancer Research Center

R01-CA-23677 New Method for Separating Leucocyte Subpopulations
John W. Parker University of Southern California

R01-CA-25612 Immunological Effects on Tumor Growth & Rejection
Janet M. Plate Rush University

R01-CA-22544 Lymphocyte Responses to Syngeneic Antigens
Nicholas M. Ponzio Northwestern University

R01-CA-24300 Functional Analysis and Regulation of Leukemic Cells
Peter Ralph Sloan Kettering Institute for Cancer Research

R01-CA-24450 T-Cell Receptor and Effector Molecules
Douglas D. Redelman University of California, San Diego

R01-CA-24443 Immunology of Germfree Genetically Thymusless Mice
Norman D. Reed Montana State University

R01-CA-21100 Immunosuppression by Adjuvant-Stimulated T Cells
Carol L. Reinisch Sidney Farber Cancer Institute

R01-CA-23887 The Behavior of Cell Suspensions In Vitro and In Vivo
Robert W. Rosenstein Yale University

R01-CA-25613 Membrane Components of Normal and Leukemic Leukocytes
Gordon D. Ross University of North Carolina

R01-CA-20823 Lymphocyte Production and Traffic in the Bone Marrow
Cornelius Rosse University of Washington

R01-CA-23891 Immunobiology of ASV Induced Primary Brain Tumors
Thomas L. Roszman University of Kentucky

R01-CA-16885 Propagation of Thymus-Derived Lymphocyte Lines
Nancy H. Ruddle Yale University

R01-CA-24034 Glucocorticoids and Lymphocyte Function
Thomas S. Ruh St. Louis University

R01-CA-28533 Mechanisms of Tumor Destruction by Immune Effectors
John H. Russell Washington University

R01-CA-23686 Macrophage-Mediated Injury Causing Tumor Regression
Stephen W. Russell University of North Carolina

R01-CA-16011 Opsonic and Phagocytic Activity During Tumor Growth
Thomas M. Saba Albany Medical College

R01-CA-24289 The Role of Oxygen Radicals on Effector/Tumor Cells
Arthur L. Sagone Ohio State University

R23-CA-28061 Autorecognition and Immunoreactivity
James W. Scheffel Sloan Kettering Institute for Cancer Research

R01-CA-25738 T Cell Differentiation: Molecular Mechanisms
Margrit P. Scheid Sloan Kettering Institute for Cancer Research

R01-CA-21539 Macrophage Regulation of Antitumor Immunocytotoxicity
Jon R. Schmidtke University of Minnesota

R01-CA-15236 Macrophage Recognition and Tumor Cell Interactions
Alan D. Schreiber University of Pennsylvania

R01-CA-22677 Pathobiology of Myeloma and Anti-Idiotypic Immunity
Hans Schreiber University of Chicago

R01-CA-22845 Immune Response to Modified Self and Tumor Antigens
David W. Scott Duke University

R01-CA-16136 The Role of Rabbit Lymphoid Cells in Tumor Immunity
Stewart Sell University of California, San Diego

R01-CA-24442 Chemical Basis for Receptor Recognition of Lysozymes
Eli E. Sercarz University of California, Los Angeles

R01-CA-22452 Monocytes and Macrophages in Resistance to Cancer
Somesh D. Sharma Palo Alto Medical Research Foundation

R01-CA-25567 Stimulation of Tumor Cell Growth by Antibody
William T. Shearer Baylor College of Medicine

R01-CA-14113 Tumor Defense by Platelets and Macrophages
Hyun S. Shin Johns Hopkins University

R01-CA-11605 Immunological Reactivity in Special Circumstances
Richard L. Simmons University of Minnesota

R01-CA-17643 Regulation of T-Cell Proliferation and Differentiation
Kendall A. Smith Dartmouth College

R01-CA-15334 Cellular Mechanisms in Tumor-Specific Immunity
Richard T. Smith University of Florida

R01-CA-21360 Subversion of Immune Mechanisms in Malignant Tumors
George L. Spitalny Trudeau Institute

R23-CA-24481 The Role of Macrophages in Tumor Growth Control
Miguel J. Stadecker Tufts University

R01-CA-27942 Role of the Spleen in the Growth of a B Cell Leukemia
Samuel Strober Stanford University

R01-CA-17004 Alterations of T Cells in Lymphoproliferative Disease
Samuel Strober Stanford University

R01-CA-17818 Tumor Immunity and Tumor-Host Interactions
Osias Stutman Sloan Kettering Institute for Cancer Research

R01-CA-21399 Binding of Bacteria to Normal & Leukemic Lymphocytes
Marius C. Teodorescu University of Illinois Medical Center

R01-CA-14462 Properties of Lymphoid Tumor Cells In Vivo and In Vitro
Geertruida J. Thorbecke New York University

R01-CA-23024 Allograft Rejection and Enhancement
Nicholas L. Tilney Harvard University

R01-CA-17733 Lymphocyte Antigens: Structure, Function and Synthesis
Ian S. Trowbridge Salk Institute for Biological Studies

R01-CA-23681 Factors Affecting Phagocyte Responses to Lipids
Stephen R. Turner Duke University

R01-CA-15960 Immunotherapy of Mammary Tumors
Jan Vaage Tufts University

R01-CA-20172 Ontogeny of Adaptive Immunity
Pierson J. Van Alten University of Illinois Medical Center

R01-CA-16652 Macrophage Functions in Tumorigenesis
William S. Walker St. Jude Children's Research Hospital

R01-CA-18893 Killing of Cancer Cells by BCG Activated Cells
Alwin H. Warfel Sloan Kettering Institute for Cancer Research

R01-CA-24430 Cyclic Nucleotides and Lymphocyte Proliferation
H. James Wedner Washington University

R01-CA-13339 Host Defense Against Lymphoblastic Leukemia
Marc E. Weksler Cornell University Medical Center

R01-CA-27599 Genetic Control of Resistance and Immunity to P815
Robert M. Williams Northwestern University

P01-CA-15822 Immunobiology of Normal and Neoplastic Lymphocytes
Darcy B. Wilson University of Pennsylvania

R01-CA-17800 Studies in Tumor Immunology
Henry J. Winn Massachusetts General Hospital

R01-CA-20044 Transplantation Immunology
Henry J. Winn Massachusetts General Hospital

R01-CA-24436 Receptor Function in Lymphocyte Differentiation
Leon Wofsy University of California, Berkeley

R01-CA-19333 Role of Macrophages in Tumor Growth and Metastasis
Gary W. Wood University of Kansas College Health Sciences & Hospital

R01-CA-17533 Myelogenous Leukemia-Associated Antigens
Carl J. Wust University of Tennessee

R01-CA-28482 Roles of Armed Monocytes in Control of Metastasis
Yasuhiro Yamamura Medical University of South Carolina

HUMORAL FACTORS OTHER THAN ANTIBODY

R23-CA-27415 Role of Mediators in Leukemic Cell Immune Responses
Robert C. Butler Arlington Hospital

R01-CA-01786 Human Blood and Tissue Proteins
Harold F. Deutsch University of Wisconsin

R01-CA-27903 Interferon as a Mediator of Cellular Immunity
Lois B. Epstein University of California, San Francisco

R01-CA-24435 Synthesis of Antibody Peptides That Fix Complement
Bruce W. Erickson Rockefeller University

R01-CA-24474 Killer Assisting Factor in Cell Mediated Lympholysis
James H. Finke Cleveland Clinic Hospital

R01-CA-26019 Isolation of Macrophage Agglutination Factor
Henry P. Godfrey State University of New York

R01-CA-24974 Chemical & Immunological Characteristics of Thymosin
Allan L. Goldstein George Washington University

R01-CA-25017 Chemical & Immunological Characteristics of Thymosin
Allan L. Goldstein George Washington University

R01-CA-28123 The Induction of Human Interferon in C-10 Cells
Ru-Chih C. Huang Johns Hopkins University

R01-CA-24476 T-Lymphocyte Differentiation
Genevieve S. Incefy Sloan Kettering Institute for Cancer Research

R01-CA-27590 Human Immune Interferon Preparation Antitumor Effect
Howard M. Johnson University of Texas Medical Branch

R01-CA-25035 Immune Interferons: Production and Role in Immunity
Roberta M. Kamin University of California, San Francisco

R01-CA-24447 Complement Attack Proteins as Lymphocyte Surface AGS
William P. Kolb University of Texas Health Sciences Center

R01-CA-24337 Tumor Suppression by Alpha-Macroglobulins of Mice
Peter H. Koo Northeastern Ohio Universities College of Medicine

R01-CA-23777 Study of Serum Factors Modifying Tumor Immunity
Robert N. Lausch University of South Alabama

R01-CA-26143 Control of Complement-Mediated Tumor Cell Cytolysis
Thomas F. Lint Rush University

R01-CA-20882 Host Cell Interaction of Animal Viruses
Philip I. Marcus University of Connecticut

R01-CA-24441 Biochemical Studies of Lymphokines and Related Agents
Manfred M. Mayer Johns Hopkins University

R01-CA-16857 Effect of Interferon Therapy on NonHodgkin's Lymphoma
Thomas C. Merigan Stanford University

R01-CA-25750 Structure-Function Relations of Immunoregulatory Protein
J. Bruce Miller University of Chicago

R01-CA-25047 Biochemical Studies of a Major Opsonic Protein
Janos Molnar University of Illinois Medical Center

R01-CA-15129 A Serum Immunosuppressive Factor in Cancer
Frederick L. Moolten Boston University

R01-CA-26051 Lymphokines Formed In Vivo: Role in Tumor Suppression
Richard G. Moulton University of Texas Medical Branch

R01-CA-26224 Mouse Tumor Assays for Lymphokine Immunotherapy
Ben W. Papermaster Cancer Research Center

R01-CA-26023 Evaluation of Thymic Hormones in Human Cancer
Yehuda Z. Patt University of Texas System Cancer Center

R01-CA-21225 Macrophage Activation by Lymphocyte Mediators
Eileen Remold-O'Donnell Center for Blood Research

R01-CA-24446 Complement Chemistry and Biology
Lennart Roden University of Alabama

R01-CA-07191 T Lymphocytes and Lymphotoxin in Tumor Immunity
Werner Rosenau University of California, San Francisco

R23-CA-27565 Tumor Cell Killing by a Soluble Macrophage Product
Somesh D. Sharma Palo Alto Medical Research Foundation

R01-CA-26842 Biological Functions of Alpha 2 Macroglobulin
Joan E. Stein-Streilein University of Texas Health Sciences Center

R01-CA-27452 Complement Membrane Attack Proteins Binding/Biosynthesis
John S. Sundsmo Trudeau Institute

R01-CA-19721 Human Interferon Production for Antitumor Studies
Yin-Hwee Tan University of Calgary

R01-CA-19529 Cell-Mediated Immunity in Humans: Mechanisms and Uses
Fred T. Valentine New York University

IMMUNODIAGNOSTIC TESTS FOR CANCER

R01-CA-16328 Polyamine Radioimmunoassay Studies in Cancer
Robert A. Campbell University of Oregon Health Sciences Center

R01-CA-24915 Lymphoid Cell Purine Nucleoside Phosphorylase
Nicholas Catsimpoolas Massachusetts Institute of Technology

R01-CA-24698 614C Arginine Pulse Labeling of Plasma Cell Neoplasms
Richard L. Humphrey Johns Hopkins University

R23-CA-27782 Enzyme Marker for Hematopoietic Cells
Sohaib A. Khan Case Western Reserve University

R01-CA-24679 Extra-nodal Lymphomas: Immunology and Ultrastructure
Daniel M. Knowles, II Columbia University

R01-CA-27690 Multiple Cell Marker Analysis in Hematopoietic Tumors
Benjamin Koziner Sloan Kettering Institute for Cancer Research

R01-CA-14413 Study of Human Lymphatic Neoplasia
Jun Minowada Roswell Park Memorial Institute

R01-CA-20364 Immunodiagnosis of Melanoma
Hilliard F. Seigler Duke University

R01-CA-23047 Cell-Mediated Immunity and Assay for Breast Cancer
Robert H. Yonemoto City of Hope National Medical Center

IMMUNOGENETICS

R01-CA-12421 Structure of Immunoglobulin Messenger RNAs and Genes
Jerry M. Adams Walter and Eliza Hall Institute Medical Research

R01-CA-22131 Immunogenetics of LY Systems
Edward A. Boyse Sloan Kettering Institute for Cancer Research

R01-CA-20473 Immunogenetics of the TLA Region of Chromosome 17
Edward A. Boyse Sloan Kettering Institute for Cancer Research

R01-CA-17680 Genetics, the Lymphocyte and Tumor Regression
Walter M. Collins University of New Hampshire

R01-CA-25038 Major Histocompatibility Complex in the Wild Rat
Donald V. Cramer University of Pittsburgh

R01-CA-23568 Immunoresponse to Human Surface Antigens
Carlo M. Croce Wistar Institute of Anatomy and Biology

R01-CA-12844 Controls of Proliferation Specific for Leukemias
Gustavo Cudkowicz State University of New York

R01-CA-24950 A Thymus Determined Mechanism of Leukemia Resistance
Syamal K. Datta Tufts University

R01-CA-24473 Genetics & Functions of (H-2 Linked) I Region
Chella S. David Mayo Foundation

P01-CA-22507 Immunogentics of the Major Histocompatibility Complex
Bo Dupont Sloan Kettering Institute for Cancer Research

R01-CA-24607 HLA Restricted Suppressor T Cells of Mixed Lymphocytes
Edgar G. Engleman Stanford University

R01-CA-23027 Immunogenetic Mapping of the Cell Surface
Lorraine Flaherty New York State Department of Health

R01-CA-20094 Molecular Genetics of Antibody Formation
Julian B. Fleischman Washington University

R01-CA-22662 Genetics and Function of Murine Ia Antigens
Jeffrey A. Frelinger University of Southern California

R01-CA-25746 Genetic Mechanisms in Immunologic Deficiency States
H. Hugh Fudenberg Medical University of South Carolina

R01-CA-17034 Immunology of Human Lymphoid Tumors of B-Cell Origin
Kazimiera Gajl-Peczalska University of Minnesota

R01-CA-15146 Genetic Control of the Immune Response
David L. Gasser University of Pennsylvania

R01-CA-25507 B Cell Expression of Immunoglobulin V and C Regions
Patricia J. Gearhart Carnegie Institution

R01-CA-18659 Chemical Genetic and Cellular Aspects of Immunogenicity
Thomas J. Gill, III University of Pittsburgh

R01-CA-15808 Genetic Markers, Leukemogenesis, and Thymic Function
Paul D. Gottlieb Massachusetts Institute of Technology

R01-CA-23770 Antigen Induced Lymphoma of Mice
Geoffrey Haughton University of North Carolina

R01-CA-04681 Genetic Studies with Mammalian Cells
Leonard A. Herzenberg Stanford University

R01-CA-23025 Comparative Transplantation Immunogenetics
William H. Hildemann University of California, Los Angeles

R01-CA-13953 Control of Myeloma Tumor Growth
Ru-Chih C. Huang Johns Hopkins University

R01-CA-13287 Genetic Basis of Antigenic Variation
Robert A. Hyman Salk Institute for Biological Studies

R23-CA-25944 Relationship of Leukemogenesis and Immune Responsiveness
David A. Johnson Jackson Laboratory

R01-CA-24263 Hybridomas: Production and Genetic Applications
Roger H. Kennett University of Pennsylvania

R01-CA-14054 Malignant Behavior and Cellular Antigen Expression
George Klein Caroline Institute

R01-CA-22958 Analysis of Idiotype Mutants
Heinz Kohler University of Chicago

R01-CA-25416 Immunogenetics of NK-1 Plus Natural Killer Cells
Gloria C. Koo Sloan Kettering Institute for Cancer Research

R01-CA-22462 Differentiative Immunogenetics: The T/T Locus
Gloria C. Koo Sloan Kettering Institute for Cancer Research

R01-CA-24438 Genetic Control of Allotype Expression of Human Ig
Stephen D. Litwin Cornell University Medical Center

R01-CA-22720 H-2 Complex and Susceptibility to Mammary Tumor Virus
Carole A. Long Hahnemann Medical College & Hospital of Philadelphia

R01-CA-21062 NK Cells in Resistance to Marrow Transplantation
Eva Lotzova University of Texas System Cancer Center

R01-CA-24497 Immunogenetic Analysis of Rous Sarcoma Development
Raymond A. McBride Baylor College of Medicine

R01-CA-26297 Primary Structure of MHC I Region Associated Antigens
David J. McKean Mayo Foundation

R01-CA-22080 Cell Surface Antigens of Lymphocytes and Tumor Cells
Ian F. McKenzie University of Melbourne

R01-CA-22948 Gene Mechanisms in Lymphoma and Lymphoproliferation
Edwin D. Murphy Jackson Laboratory

R01-CA-23882 Defective Self-Recognition as a Cause of Cancer
John R. Neefe Georgetown University

R01-CA-22512 Viral Leukemia Therapy by Resistant Tissue Transplant
James P. Okunewick Allegheny-Singer Research Corporation

P01-CA-21112 Clinical and Basic Studies of Plasma Cell Dyscrasias
Elliott F. Osserman Columbia University

R01-CA-24440 Recessive Genes Controlling Immunocompetence
M. Boris Rotman Brown University

R01-CA-22241 Lymphocyte Subsets in Immune-Deficiency States
Margrit P. Scheid Sloan Kettering Institute for Cancer Research

R01-CA-16367 Lymphocytes-3Gene Expression and Activation Events
Stewart Sell University of California, San Diego

R01-CA-18640 Behavior of Weak Transplantation Antigens
Willys K. Silvers University of Pennsylvania

R01-CA-22664 Somatic Cell Hybridization of Immune Cytolytic T-Cells
Geronimo Terres, Jr. Tufts University

R01-CA-23026 The Major Histocompatibility Complex of the Rabbit
Robert G. Tissot University of Illinois Medical Center

R01-CA-22268 Genetic Aspects of Tumor Host Immune Relationship
Noel L. Warner University of New Mexico

R01-CA-22105 Allotypes of Murine Immunoglobulins
Noel L. Warner University of New Mexico

R01-CA-25139 Study of Group 5 Antigens in Hematologic Malignancies
Reed P. Warren Fred Hutchinson Cancer Research Center

R01-CA-10097 Cellular Antigens of Normal and Malignant Rat Tissues
Peter J. Wettstein Wistar Institute of Anatomy and Biology

R01-CA-20107 B Cell Alloantigens, Molecular Basis and Disease Aspects
Robert J. Winchester Rockefeller University

R01-CA-20105 Information from Immunological Reaction Tables
Andrew R. Wohlgemuth University of Maine

R01-CA-17481 Mechanisms of Allograft Tolerance
Peter W. Wright Fred Hutchinson Cancer Research Center

R01-CA-23676 Immunogenetics of Self-Identification
Kunio Yamazaki Sloan Kettering Institute for Cancer Research

R01-CA-20531 Genetic Analysis of Normal and Malignant Lymphocytes
Edmond J. Yunis Sidney Farber Cancer Institute

IMMUNOLOGY PROGRAM

R01-CA-25945 Mechanisms of Tumor Regression by Hapten Treatment
Kazuhiko Arai University of Pennsylvania

R01-CA-26386 Monoclonal Antibody Therapy of Cancer
Irwin D. Bernstein Fred Hutchinson Cancer Research Center

R01-CA-26053 Inflammatory Autoimmune Responses and Neoplasia
Pierluigi E. Bigazzi University of Connecticut Health Center

P01-CA-25863 Control of Neoplasia by Passive Serum Therapy
Dani P. Bolognesi Duke University

R01-CA-26480 Antitumor Activity of Tumor-Bearer Lymphoid Cells
Sheldon Dray University of Illinois Medical Center

R01-CA-25965 Study of Micrometastasized Stable Murine Tumor Clones
Vithal K. Ghanta University of Alabama

R01-CA-25214 Immunological Studies of Herpes Induced Fibrosarcoma
John P. Gusdon, Jr. Wake Forest University

R01-CA-25854 Purified Homologous Antibody: Carrier for Isotopes
Paul Lo Gerfo Columbia University

R01-CA-26286 Polymerized Tumor Antigen-Adjuvant Immunotherapy
James A. Neidhart Ohio State University

R01-CA-26162 Mechanism of Nanaase-Enhanced Tumor Immunogenicity
Jack H. Pincus SRI International

R01-CA-24936 Bioassay for Antitumor Effects of R.E. Stimulants
Julian W. Proctor Allegheny-Singer Research Corporation

R01-CA-15952 Experimental Studies on Immunity to Cancer Cells
Arnold E. Reif Boston City Hospital

R01-CA-27772 In Vitro Immunization to Human Tumor Cells
Brahma S. Sharma University of California, Irvine

R01-CA-16869 FOCMA and Its Therapeutic Application
Gordon H. Theilen University of California, Davis

R01-CA-26245 Mechanisms of Successful Adoptive Immunotherapy
Robert L. Truitt Mount Sinai Medical Center

IMMUNOPROPHYLAXIS AGAINST CANCER

R01-CA-28060 Immunobiology Metastasis
Philip Frost University of California, Irvine

IMMUNE STATUS

R01-CA-23709 Induction of Tolerance and Suppressor Cells In Vitro
Frank L. Adler St. Jude Children's Research Hospital

R01-CA-24873 Immunosuppression in Cancer Patients
Arthur D. Bankhurst University of New Mexico

R01-CA-22141 Protein SAA in Neoplastic Disease
Merrill D. Benson Indiana University-Purdue University

R01-CA-11898 Etiology, Immunology and Biology of Brain Tumors
Darell D. Bigner Duke University

R01-CA-25165 Specific Immunity and Prognosis in Breast Cancer
Maurice M. Black New York Medical College

R01-CA-23500 Prostaglandins and Immunosuppression in Cancer
Richard S. Bockman Sloan Kettering Institute for Cancer Research

R01-CA-26169 Immunosuppression During Acute Avian Leukemia
Henry R. Bose, Jr. University of Texas

R01-CA-24244 Studies of the Immunobiology of B-16 Melanoma
Neil I. Brody Downstate Medical Center

R01-CA-18600 Masking of Antigens at Cancer Cell Surfaces
John F. Codington Massachusetts General Hospital

R01-CA-19754 Immunoselection and Cancer: A Problem in Evolution
Melvin Cohn Salk Institute for Biological Studies

R01-CA-22518 Suppressor Defect, Autoimmunity and Neoplasia
Michael J. Dauphinee University of California, San Francisco

R01-CA-25917 Cellular and Genetic Aspects of Antitumor Immunity
Raymond A. Daynes University of Utah

R01-CA-26268 Anti-II Antibodies and II Antigens in Carcinomas
Volker E. Dube Evanston Hospital

R01-CA-20816 The Pathogenesis of Autoimmunity in New Zealand Mice
M. Eric Gershwin University of California, Davis

R01-CA-24362 Leukemogenesis in Gnotobiotic Nude Mice
M. Eric Gershwin University of California, Davis

R01-CA-26861 Determination of Malignant Potential of Cultured Cells
Beppino C. Giovanella Stehlin Foundation for Cancer Research

R01-CA-23648 Immunity to Human Ovarian Tumors and Chemotherapy
John S. Haskill University of North Carolina

R01-CA-14300 Mechanism of Immunosuppression by Plasmacytomas
Helga F. Havas Temple University

R01-CA-19148 Lymphocyte Allogeneic Inhibition and Tumor Immunity
Karl E. Hellstrom Fred Hutchinson Cancer Research Center

R23-CA-24875 Immune Recognition of Autologous Renal Cell Carcinoma
George P. Hemstreet, III University of Alabama

R01-CA-27437 Immune Reactivity and Mammary Neoplasia
Gloria H. Heppner Michigan Cancer Foundation

R01-CA-17673 Regulation of Immunity by Antibody and B-Cells
Michael K. Hoffmann Sloan Kettering Institute for Cancer Research

R01-CA-26447 Pathogenesis of Preleukemic Aplastic Anemia
Edward A. Hoover Ohio State University

R01-CA-26882 Antibody Responses of Tumor Bearers to Their Tumors
Paul A. Klein University of Florida

R01-CA-27701 Human Immunoregulatory Gangliosides
Stephan Ladisch University of California, Los Angeles

R23-CA-25932 Thymic Antigens, Tolerance and Self-Recognition
Edmund C. Lattime Sloan Kettering Institute for Cancer Research

R01-CA-25039 Control of Immunosuppressor Cell Activation Function
Elinor M. Levy University Hospital

R01-CA-25641 Effect of Cancer on Human Monocyte Cytotoxic Mechanisms
Albert F. Lo Buglio University of Michigan

R23-CA-29155 Stress and Human Cell-Mediated Immunity
Steven Locke Beth Israel Hospital

R01-CA-18185 Hereditarily Athymic--Asplenic Mice: Tumor Growth
Bismarck B. Lozzio University of Tennessee

R01-CA-24725 Factors Negating Response to Mammary Adenocarcinoma
Zoltan J. Lucas Stanford University

R01-CA-24511 Auto-Regulation of Immune Response by Anti-Idiotype
George L. Mayers Roswell Park Memorial Institute

R01-CA-25765 Effects of Therapy on Tumor-Specific Immunity
Malcolm S. Mitchell University of Southern California

R01-CA-26827 C-Reactive Protein Regulation of Tumor Immunity
Richard F. Mortensen Michigan Cancer Foundation

R01-CA-23353 Stimulatory Effect of Immunization on Tumorigenesis
Donna M. Murasko Medical College of Pennsylvania

R01-CA-25041 Murine T Lymphocyte Receptors for Alloantigens
John R. Neefe Georgetown University

R23-CA-26116 Antigen-Specific Suppression of Antitumor Immunity
Karen A. Nelson Fred Hutchinson Cancer Research Center

R01-CA-22517 Monocyte Function in Neoplasia
Sigurd J. Normann University of Florida

R01-CA-24901 Immunomodulation of Oncogenesis
Henry C. Outzen Jackson Laboratory

R01-CA-26144 Leukocyte Adherence Inhibition Assay in Human Cancer
Arnold E. Powell Case Western Reserve University

R01-CA-20920 Mechanisms of Carcinogenesis
Richmond T. Prehn Jackson Laboratory

R01-CA-23561 Immunopathology X-Linked Lymphoproliferative Syndrome
David T. Purtilo University of Massachusetts Medical School

R01-CA-25072 Suppressor T-Cells in Malignant Lymphoma
Peter C. Raich West Virginia University

R01-CA-20543 Antigen-Antibody Complexes in Cancer Patients' Sera
Roger D. Rossen Baylor College of Medicine

R01-CA-23809 Natural and Tissue-Specific Immunity to Human Neoplasms
Eero J. Saksela University of Helsinki

R01-CA-10018 Experimental Model of Malignant Lymphoma
Robert S. Schwartz New England Medical Center Hospital

R01-CA-20408 Immunodeficiency and Tumorigenesis
Leonard D. Schultz Jackson Laboratory

R01-CA-18149 Autoimmunity and Neoplasia in New Zealand Black Mice
Benjamin V. Siegel University of Oregon Health Sciences Center

R01-CA-18987 Cell Biology of Mammary Gland Neoplasia
Glenn L. Slemmer University of British Columbia

R01-CA-27390 Ethylnitrosourea-Induced Rat Gliomas
Alexander M. Spence University of Washington

R01-CA-20091 Immunological Enhancement of Renal Allografts
Frank P. Stuart University of Chicago

R01-CA-15988 Immune Surveillance and Cancer
Osias Stutman Sloan Kettering Institute for Cancer Research

R01-CA-25686 Epstein-Barr Virus: Effects on Human Lymphocytes
Ciro V. Sumaya University of Texas Health Sciences Center

R01-CA-26799 Immunological Reactivities and MTV Infections
Aldo Tagliabue Mario Negri Institute Pharmacologic Research

R01-CA-15333 Rheumatoid Factor and Tumor-Host Interaction
Jeremiah J. Twomey Baylor College of Medicine

R01-CA-18995 Studies of Tumor Dormancy and Emergence
E. Frederick Wheelock Thomas Jefferson University

R01-CA-12461 Suppression of Established Leukemia Virus Infections
E. Frederick Wheelock Thomas Jefferson University

R01-CA-24429 Immunosuppressants and Lymphocyte Function
Alan Winkelstein Montefiore Hospital

R01-CA-26141 Cell-Mediated Cytotoxicity in Children with Neoplasms
Alice L. Yu University of California, San Diego

R01-CA-21672 Immunologic Factors in Spontaneous Mammary Carcinoma
Edmond J. Yunis Sidney Farber Cancer Institute

R01-CA-15585 A Soluble Mediator of Tumor-Induced Immunosuppression
Susan B. Zolla-Pazner New York University

LYMPHOCYTE BIOLOGY

R01-CA-28099 F.A.C.S. of Immunologic Components
D. Bernard Amos Duke University

R01-CA-26695 Antigen-Specific T-Cell Clones: Generation and Analysis
Harvey I. Cantor Sidney Farber Cancer Institute

R01-CA-26284 Regulation of Adenosine Deaminase in Human Cells
Peter E. Daddona University of Michigan

R01-CA-26257 Modulation of Lymphocyte Cytolysis of Tumor Cells
Ernest W. Fuson University of Tennessee

R01-CA-08593 Immune Response to Tumor Grafts
Richard K. Gershon Yale University

R01-CA-26369 T-MICG and N-MICG in Lymphoid Malignancy
Stephen P. Hauptman Thomas Jefferson University

R23-CA-26223 Proteinases of Human Natural Killer Cells
Dorothy Hudig University of California, San Diego

P01-CA-25803 Control of Normal and Abnormal Cell Development
David H. Katz Scripps Clinic and Research Foundation

R01-CA-26775 Anatomical Gradient in Susceptibility to Oncogenesis
Richmond T. Prehn Jackson Laboratory

R01-CA-27326 Cancer Development of the Idiotype Network
Hans Schreiber University of Chicago

R01-CA-26467 Effector and Suppressor Mechanisms of Tumor Immunity
Robert D. Stout Brandeis University

R23-CA-27115 Characterization of Natural Killer Cells
Anna Tai University of New Mexico

R01-CA-26344 Autologous Lymphocyte Reactions and Immune Surveillance
Marc E. Weksler Cornell University Medical Center

R01-CA-26760 Monocyte-Lymphocyte Interactions in Mycosis Fungoides
William L. Weston University of Colorado Medical Center

R01-CA-26738 Cellular Immunity to Tumors
Joyce M. Zarling University of Minnesota

MACROPHAGE BIOLOGY

R01-CA-26824 Mononuclear Phagocytes in Human Ovarian Carcinoma
Alberto Mantovani Mario Negri Institute Pharmacologic Research

R01-CA-26504 Regulation of Granulocyte and Macrophage Production
Evan R. Stanley Yeshiva University

R23-CA-26158 The Immunogenetics of Macrophage Suppression in Man
Stephen V. Stux Sidney Farber Cancer Institute

R01-CA-24916 Studies of a Tumor Factor That Affects Macrophages
Gnanasigamoni Sundharadas University of Wisconsin

R01-CA-20819 Phagocytic Cells: Regulation, Dysfunction and Disease
Dennis E. Van Epps University of New Mexico

R01-CA-27070 Macrophage Tumor Cell Killing and Red Cell Catabolism
Joe B. Weinberg Duke University

IMMUNE SURVEILLANCE

R01-CA-26782 In Vivo Role of Natural Killer Cells
Rolf Kiessling Caroline Institute

R01-CA-20833 Cell-Mediated Cytotoxicity in Humans
Giorgio Trinchieri Wistar Institute of Anatomy and Biology

R01-CA-26752 On the Relevance and Functions of Natural Killer Cells
Hans L. Wigzell University of Uppsala

CANCER IMMUNOTHERAPY IN ANIMAL MODELS AND IN VITRO STUDIES

R23-CA-24911 Studies of Tumor-Specific Immune Responses
Amnon Altman Scripps Clinic and Research Foundation

R01-CA-15369 Immunobiology of Viral Leukemia and Its Treatment
Michael Bennett Boston University

R01-CA-28701 Chronic Graft-Versus-Host Disease in Radiation Chimera:
William E. Beschorner Johns Hopkins University

R01-CA-20484 Specific Adoptive Immunotherapy of AKR Leukemia
Mortimer M. Bortin Mount Sinai Medical Center

R01-CA-23678 Tolerance of Bone Marrow Grafts
Robert E. Click University of Minnesota

R01-CA-27579 Use of Syngeneic Hybrid Cells in Leukemia Therapy
Edward P. Cohen University of Illinois Medical Center

R01-CA-24729 Comparative Testing of Vaccines from C. parvum
Cecil S. Cummins Virginia Polytechnic Institute and State University

R01-CA-17467 Adoptive Immunotherapy of Cancer by Allogeneic Marrow
Eugene E. Emeson Montefiore Hospital and Medical Center

R01-CA-10777 Adoptive Chemoimmunotherapy of Tumors in Mice
Alexander Fefer University of Washington

R01-CA-22267 Immunobiology of Bone Tumors and Bone Allografts
Gary E. Friedlaender Yale University

R01-CA-16699 A Model for Multiple Modality Therapy of Osteosarcoma
Raymond N. Hiramoto University of Alabama

R01-CA-23602 Bone Marrow Transplantation After LY Subset Elimination
Susan E. Krown Sloan Kettering Institute for Cancer Research

R01-CA-23372 Studies on Soluble Antigens on MTV Induced Mammary Tumor
Calbert A. Laing Howard University

R23-CA-25486 Generation of Effector Cells for Tumor Immunotherapy
Takashi Maki New England Deaconess Hospital

R01-CA-23865 Transfer of Tumor Immunity with Immune-RNA
John A. Mannick Peter Bent Brigham Hospital

R01-CA-21099 Antitumor Activity of BCG, C. parvum and Glucan
Robert Megirian Albany Medical College

R01-CA-19318 Immunotherapy of Experimental Brain Tumors
Robert A. Morantz University of Kansas College Health Sciences & Hospital

R01-CA-16642 Immunological Basis of Tumor Regression
Robert J. North Trudeau Institute

R01-CA-24368 Immunobiology of Early Lymphocyte Development
Vicki L. Sato Harvard University

R01-CA-20526 New Approaches to Immunotherapy
Robert J. Scibienski University of California, Davis

R01-CA-22513 Minimal Residual Disease After Rat Colon CA. Excision
Glenn D. Steele, Jr. Sidney Farber Cancer Institute

R01-CA-28498 Tumor Immunotherapy with Pharmacological Agents
Robert L. Stolfi Catholic Medical Center Brooklyn Queens Nursing

R01-CA-21790 Antitumor Activity of Glycolipid-Endotoxin Mix
Kuni K. Takayama University of Wisconsin

R01-CA-20744 The Immunogenicity of Tumor-Macrophage Hybrids
David W. Talmage University of Colorado Medical Center

R01-CA-18047 Irradiation and Marrow Transplantation in Large Animals
E. Donnall Thomas University of Washington

P01-CA-18029 Adult Leukemia Research Center
E. Donnall Thomas Fred Hutchinson Cancer Research Center

R01-CA-24377 Cellular Immune Responses to Syngeneic Tumors
Sei Tokuda University of New Mexico

R01-CA-03367 Immunogenetic Resistance to Lymphoma-Leukemia
John J. Trentin Baylor College of Medicine

R01-CA-18440 Germfree Techniques Applied to Adoptive Immunotherapy
Robert L. Truitt Mount Sinai Medical Center

R01-CA-18105 Immunotherapy Studies of Spontaneous Malignancy
Paul L. Weiden Fred Hutchinson Cancer Research Center

R01-CA-23469 Cells Involved in Spontaneous Regression of Tumors
Tsu-Ju T. Yang University of Connecticut

R01-CA-16342 Immunoprophylaxis and Immunotherapy of Lung Cancer
Bruce S. Zwilling Ohio State University

CLINICAL CANCER IMMUNOTHERAPY

P01-CA-15147 Leukemia--Lymphoma Program
Stanley P. Balcerzak Ohio State University

R01-CA-28740 Specific Immunotherapy with Monoclonal Antibodies
Robert C. Bast, Jr. Sidney Farber Cancer Institute

P01-CA-23175 A Program in Bone Marrow Transplantation
Robert P. Gale University of California, Los Angeles

P01-CA-23766 Marrow Transplantation in Aplastic Anemia & Leukemia
Robert A. Good Sloan Kettering Institute for Cancer Research

R13-CA-27210 Thirteenth International Cancer Congress
William B. Hutchinson International Union Against Cancer

R01-CA-13599 Relation of Immune Reactions to Cutaneous Neoplasia
Edmund Klein Roswell Park Memorial Institute

R01-CA-13456 An Integrated Clinical Program for Melanoma
Michael J. Mastrangelo Institute for Cancer Research

P01-CA-12582 Multidisciplinary Cancer Diagnosis and Therapy
Donald L. Morton University of California, Los Angeles

R01-CA-23338 Intratumoral Immunotherapy Prior to Surgery
James P. Neifeld Virginia Commonwealth University

R01-CA-28696 Experimental Therapy of Human Melanoma with Vaccines
Herbert F. Oettgen Sloan Kettering Institute for Cancer Research

P01-CA-19267 Clinical Immunobiology Program Project
Herbert F. Oettgen Memorial Hospital for Cancer-Allied Diseases

R01-CA-25740 Lymphokine Mediated Tumor Immunotherapy
Ben W. Papermaster Cancer Research Center

P01-CA-15396 Bone Marrow Transplantation in Human Disease
George W. Santos Johns Hopkins University

P01-CA-18221 Marrow Grafting in Treatment of Hematologic Malignancies
Rainer F. Storb Fred Hutchinson Cancer Research Center

R01-CA-25307 Study of Bacterial Component Immunotherapy in Cancer
Gerald J. Vosika University of Minnesota

STUDIES OF MECHANISMS INVOLVED IN CANCER IMMUNOTHERAPY

R01-CA-19170 Mechanisms of BCG-Mediated Suppression of Tumor Growth
Irwin D. Bernstein Fred Hutchinson Cancer Research Center

R01-CA-12102 Surgical Management of the Regional Lymph Node
Bernard Fisher University of Pittsburgh

R01-CA-17013 Lymphocyte Sensitization to Tumor Antigens In Vitro
Sidney H. Golub University of California, Los Angeles

R01-CA-14364 Selective Stimulation of Cell-Mediated Cancer Immunity
Robert L. Hunter University of Chicago

R01-CA-18647 Specific Antitumor Activity by "Armed" Lymphoid Cells
Sylvia B. Pollack Fred Hutchinson Cancer Research Center

R01-CA-21367 Mechanism of Tumor Abrogation by Glycolipid Al
Zulema R. Reggiardo University of Maryland

R23-CA-25801 Effects of Retinoic Acid on the Immune System
Neil Sidell University of California, Los Angeles

CONTRACT RESEARCH SUMMARY

Title: Molecular Studies of T Cell-mediated Cytotoxicity

Principal Investigator: Dr. Carol C. Whisnant
Performing Organization: Duke University
City and State: Durham, NC

Contract Number: NO1-CB-74152
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Study mechanisms of T cell-mediated cytotoxicity at the molecular level.

Approach: Use as a model system the specific killing of allogeneic tumor cells by immune peritoneal exudate cells (CHY and EL-4 tumor cell lines). Spin labeling will be applied to studying cell interactions.

Progress: The contractor's previous work has shown that, although the murine lymphomas EL4 and CHY bind equivalent amounts of alloantibody per unit of surface area, alloimmune cytotoxic T lymhocytes bind specifically to CHY to a lesser extent than to EL4. They have begun a series of experiments using partially purified, detergent soluble H2 antigens from EL4 and CHY to determine (1) whether these differences in recognition by antibody and cytotoxic T lymphocytes persist when the target H2 molecules are removed from their respective plasma membranes, into the plasma membrane of CHY tumor cells. For H2 antigen preparations from CHY and EL4 they are comparing serologically detectable activity per weight of protein with the amount of protein which will bind to antigen receptors on cytotoxic T lymphocytes. H2 antigens have purified from EL-4 tumor cells to a large extent and a radioimmunoassay has been developed for quantitating serologically detectable H2 antigen. Currently, binding of soluble H2 antigen to receptors on cytotoxic T lymphocytes is quantitated by inhibition of specific conjugate formation. A procedure for directly measuring the binding of purified and radiolabeled H2 antigens from CHY and EL4 is being developed. Studies on the insertion of H2 antigens from one cell line into the plasma membrane of another cell line are being carried out in collaboration with Dr. Peter Cresswell.

Significance to Cancer Research: Elucidation at the molecular level of mechanisms of cell-mediated cytotoxicity may suggest reasons why some tumors are resistant to such effector function and may facilitate manipulation and modification of these processes.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Characterization of Antigen-binding T-cell Receptors

Principal Investigator: Dr. P.H. Krammer
Performing Organization: Institute for Immunology
City and State: Heidelberg, Germany

Contract Number: NO1-CB-74179
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To test for the expression of alloantigen receptors on murine T-cell tumors.

Approach: Use AKR/J lymphomas; develop sensitive assay systems for alloantigen binding T-cell receptors; obtain sufficient quantities of receptor material from positive tumors to perform biochemical analyses.

Progress: Microassays in Teraski plate hanging drop cultures have been standardized for proliferative as well as cytotoxic T cell responses. These assays allow a rapid screening of a large number of T cell tumors and continuous lines of normal cytotoxic T cells for receptor expression. The specificity of two idiotype systems were established: (a) anti-idiotypic antisera made in (AKRxC57Bl/6)F1 mice against AKR anti-C57Bl/6 MLR activated T cell blasts recognizing AKR T cell receptors for C57Bl/6 alloantigens, and (b) anti-idiotypic antisera made in syngeneic AKR mice against AKR anti-TNP activated T cell blasts (containing AKR anti-TNP H-2 restricted cytotoxic T cells) recognizing idiotypes on T cell receptors of AKR cells cytotoxic for syngeneic TNP coupled target cells. The latter system provides an entirely new tool to detect idiotypes on receptors of H-2 restricted cytotoxic T lymphocytes. The F1 anti-(AKR-anti-C57Bl/6) system was employed to test AKR T cell lymphomas for alloantigen receptor expresssion. Twelve (12) different antisera were tested on 11 different tumors and no tumor was found which could be called receptor positive with certainty. Therefore, strategy was altered somewhat and a large number of continuously growing (in T cell growth factor containing medium) AKR T cell lines were established from cultures of AKR T cells stimulated against the hapten TNP. They are presently selecting the TNP specific cytotoxic T cell lines which will be tested for idiotype expression by our AKR anti-(AKR-anti-AKR-TNP) anti-idiotypic reagents in microscreening assays.

Significance to Cancer Research: T cells play a decisive role in tumor defense. The recognition process of the T cell in general and the recognition of tumor targets in particular are not understood at all. One clue to this understanding is the biochemical nature of the T-cell antigen recognition system.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Susceptibility to Tumors

Principal Investigator: Dr. Chen K. Chai
Performing Organization: Jackson Laboratory
City and State: Bar Harbor, ME

Contract Number: NO1-CB-74181
Starting Date: 9/30/77 Expiration Date: 9/29/80

Progress: The results to date show that, on the C57BL/6 genetic background, $\underline{A^y a}$ mice produced, on the average, more tumors than mice of any other tested genotypes at the agouti locus. Differences between that for $\underline{A^y a}$ and any of the other genotypes are highly significant. In the C57BL/6 x A/J crosses, including F_1, F_2, and first backcross generations, mice of the yellow genotypes produced on the average no more tumors than the non-yellow mice. However, a larger dispersion in the distribution of mice with a different number of tumors is shown in the segregation generation than in the non-segregation generation in each cross, when the data of the yellow and non-yellow mice are pooled. Especially, a trimodel distribution in the F_2 of the cross involving the $\underline{A^{Iy}}$ appeared. This suggests that two major genes affecting tumor suscepibility are segregating. These genes, present in the A/J, overshadow the effects of the $\underline{A^y}$ gene. In response to urethane, mouse strains with genetic differences between them in leukocyte levels, show differences in lung tumor production at different ages following the urethane injection. Similarly, preliminary results show that mouse strains, with genetic differences between them in thyroid activity, show differences in number of lung tumors produced. We may tentatively conclude that $\underline{A^y}$ and, perhaps, two genes of the A/J mice have a significant effect on the cell transformation for lung tumor development. All the yellow genes at the agouti locus cause the development of obesity at an early age, but only the lethal yellow $\underline{A^y}$ has a significant effect on lung tumor development. Obesity, generally considered to be a factor in favor of tumor development, is not suggested as far as the present data is concerned. However, genes either of a mutant form, as $\underline{A^Y}$, or in the polymorphic form, such as those present in A/J, affect tumor development. This implies a genetic basis for variations in tumor resistance and susceptibility between individuals in normal populations including that of man.

Significance to Cancer Research: To determine the role that a gene, or a specific genetic locus, plays in cancer development. The results of this investigation would specifically concern the mutation theory of neoplasm.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Studies on Macrophage Activation

Principal Investigator: Dr. Emil Skamene
Performing Organization: Montreal General Hospital
City and State: Montreal, Canada

Contract Number: NO1-CB-84269
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: Study the interactions of T cells for their products with macrophages that lead to activation of macrophages for reaction against tumor cells.

Approach: Investigate the identity and function of cell populations that control macrophage activation in the development of antilistereal resistance and determine the cellular level of phenotypic expression of genetically controlled high or low levels of antilistereal immunity.

Progress: The work of the past year has focused upon the differences in macrophage response between strains which are resistant versus sensitive to Listeria infection. A number of significant observations have been made. Strains of mice resistant to infection (e.g. B.10.A) show a greater cellularity in their bone marrow than sensitive strains which may reflect a larger pool of promonocytes. The exact stage of differentiation of these cells is unclear but the plating efficiency of the marrow cells from the resistant mice was lower than the cells from sensitive mice. Additional data indicates that this reflects a more rapid differentiation toward mature effector cells in the resistant animals. Utilizing soluble factors such as monocytosis-producing activity (MPA), data were developed that that the sensitive strains are deficient in their ability to mobilize as well as produce effector monocytes in the early stages of infection.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $104,900

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Bone Marrow Transplantation

Principal Investigator: Dr. Ross S. Basch
Performing Organization: New York University Medical Center
City and State: New York, NY

Contract Number: NO1-CB-74176
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To develop an animal model for transplantation of selected bone marrow cells of defined function and developmental potential.

Approach: To study the relationship between the hematopoietic precursors of immunocompetent T cells and the "natural killer cells" of bone marrow and spleen in mice.

Progress: During the past year, the contractor has continued studying the relationship of "natural killer" (NK) cells to hematopoietic stem cells. NK cells share with thymocytes sensitivity to: anti-Thy-1 antisera (monoclonal), anti-mouse brain sera, and anti-Lyt 10. All of these sera also kill prothymocytes and a subset of cortical thymocytes. Anti-RLθ1-4 (an autoantithymocyte antibody of BALB/c mice) which also kills these cells fails to eliminate NK activity. Treatment of preparations of NK cells with thymopoietin produces a 30-60% decrease in the number of lytic units detected, further indicating that these cells have some relationship to the T-cell lineage. However, the cells which rosette with NK sensitive targets (RLθ1) do not contain the enzyme TdT as measured by immunofluorescence. Since this enzyme is characteristic of the majority of bone marrow thymocyte precursors, it seems unlikely that NK cells, if they are in the thymocyte lineage, have differentiated as far as the immediate precursors of thymocytes.

Significance to Cancer Research: If natural resistance to malignancy is mediated by an immature member of the T-cell lineage, it may be possible to influence the rate of passage of cells through this compartment and alternately influence the magnitude of natural response.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
jTechnical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunogenicity of "Spontaneous" Animal Tumors

Principal Investigator: Dr. Gerald Bartlett
Performing Organization: Pennsylvania State University
City and State: Hershey, PA

Contract Number: NO1-CB-64039
Starting Date: 6/16/76 Expiration Date: 6/15/80

Goal: To test whether "spontaneous" murine tumors appear to be nonantigenic due to processes which are known to interfere with cell-mediated cytotoxicity.

Approach: Obtain new tumors likely to be nonimmunogenic ("spontaneous" or induced by means not associated with strong tumor antigens). Test immunogenicity of the tumors in sensitive in vivo tests (immunization-challenge, Winn assay). Determine the effect on immunogenicity of procedures intended to: (1) deplete suppressor activity; (2) remove blocking factors; or (3) "reactivate" sensitized cells.

Progress: The immunogenicity test on hepatoma SHB-91 has been completed. Although sc growth of tumor did not reveal any immunity to challenge, mice pretreated with irradiated normal liver or with BCG survived longer than control, untreated mice; pretreatment with irradiated tumor cells did not induce any prolonged survival after challenge. Several more experiments have been done to determine if suppressor-depleting treatments, such as adult thymectomy, splenectomy or CY pretreatment, would improve immunity to spontaneous, nonimmunogenic tumors. The pretreatments did occasionally delay growth of challenge tumors in nonimmunized mice, but that was not accompanied by better immunity after immunizing treatments. Leukemias SLC-42 and SLH-76 both disseminate via blood within 10 to 14 days after injection of cells id. Mice were treated with one of several CY doses given one day after surgical excision of the id injection-site tumor nodules. Survival of SLC-42 bearing mice was significantly prolonged only by a dose of 300 mg/kg, while doses as low as 50 mg/kg delayed death of SLH-76 bearing mice. SLC-42 immunized BALB/c mice against a CDF1 skin graft, but CDF1 spleen cells could not immunize BALB/c mice against SLC-42. Hybrids of BALB/c with C57B1/6 or with C3H/He showed natural resistance to SLC-42, but the resistance was not consistently increased by immunizing procedures.

Significance to Cancer Research: Apparently, the immunity that can be detected in vitro is ineffectual for tumor rejection in vivo. This project is to examine the role of several processes which might be interfering with tumor rejection immunity. Such information would guide subsequent efforts to improve methods for immunotherapy of cancer.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Facility for Supplying Immune-related Cell Lines

Principal Investigator: Dr. Melvin Cohn
Performing Organiztion: The Salk Institute
City and State: San Diego, CA

Contract Number: NO1-CB-23886
Starting Date: 6/26/72 Expiration Date: 2/25/81

Goal: To supply the scientific community with immune-related lines important in the study of tumor immunology and to increase the library of useful lines as they are characterized.

Approach: Since this is essentially a contract to supply cell lines, little experimental work is involved. However, the general thrust of the laboratory involved the isolation of immune-related lines of the thymus and bone marrow-derived lineages by the use of leukemia viruses, cell fusions, and chemical carcinogens. As new lines become available, they are introduced into the contract catalog.

Progress: The contractor has shipped 570 tissue culture and tumor lines to investigators in the scientific community over the past contract year. The catalog contains detailed characterization of the cell lines. This contract continues to be an excellent research resource increasingly utilized by scientists all over the world. Computerization of inventory and shipments has greatly increased the efficiency of the project.

Significance to Cancer Research: The lines in this contract are used by hundreds of laboratories for studies on tumor-specific antigens, antibody structure, immune-related cell functions, somatic genetics, and cell fusions. All of these subjects are major categories under the NCI Cancer Research Program, and the cell lines from this laboratory are key tools in these investigations.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $120,710

CONTRACT RESEARCH SUMMARY

Title: Hybridization Techniques to Obtain Functional T Cells

Principal Investigator: Dr. Harvey Cantor
Performing Organization: Sidney Farber Cancer Institute
City and State: Boston, MA

Contract Number: NO1-CB-74147
Starting Date: 9/16/77 Expiration Date: 4/30/80

Goal: Produce continuously propagatable lines of antigen-specific T cell subclasses, each expressing a distinct immune function.

Approach: Separation of T cells into functionally distinct sets; generate highly purified antigen-reactive T cells from these subsets; induce propagatable, cloned cell lines following hybridization with appropriate T cell tumors.

Progress: Analysis of the molecular basis of the specificity and function of different T cell sets has not been possible because methods for obtaining large amounts of homogeneous, antigen-specific, functional T cells are not available. "T cell" lymphomas have not proved as useful as myelomas for this purpose. Continuous activation of T cells by supernatants of Con A stimulated spleen cells have not resulted in efficient growth of antigen-specific T cell clones that secrete relevant molecules in significant amounts. "Hybrids" formed after fusion of T cells to T cell tumors rarely develop and maintain specific T cell function (perhaps due to continuous chromosome loss). Moreover, greater than 95% of the proteins secreted by "positive" hybridomas (4/360 tested) are products of the tumor cell partner. A general method has been developed for producing continuously propagatable clones of T cell sets that produce large amounts of homogeneous polypeptides that bind to antigen and mediate one or another T cell function. The procedures does not require hybridization to tumor cells. It is based upon the finding that (a) each set of T cells reuires different physiologic signals to potentiate in vitro growth and maintain antigen-specific regulatory function and (b) long-term stimulation of unselected T cell suspensions results in T inducer:T suppressor interations that inactivate specific function after a short period of time. This technique allows (a) preselection of the particular T cell set to be studied (e.g., TL^+ thymocytes, suppressor precursor, suppressor effector, and inducer T cell sets), (b) preselection of the specificity of the T cell set that is to be clonally expanded and (c) generation of large numbers of continuously propagatable clones of non-transformed cells that are specialized to synthesize and secrete functionally active, antigen-specific polypeptides which represent between 5-10% of the total proteins synthesized by these T cell clones, and greater than 80% of the total secreted proteins.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: *In Vitro* Induction of Functional Differentiation of T Cells in Tumor Immunology Systems

Principal Investigator:	Dr. Samuel D. Waksal
Performing Organization:	Tufts University
City and State:	Boston, MA

Contract Number: NO1-CB-74150
Starting Date: 9/16/77 Expiration Date: 9/15/80

Goal: Study the role of thymus epithelium in the differentiation and transformation of lymphocytes, including isolation and characterization of prothymocytes from normal, athymic, and tumor-bearing mice.

Approach: Presentation of tumor and virus antigens to prothymocytes during *in vitro* differentiation to produce populations of antitumor effector cells. Analysis of the ability of thymus epithelium to induce T lymphocyte differentiation in high leukemic strains (AKR/J and HRS/J) as opposed to control strains.

Progress: The group is studying the cellular, immunological, and virological events that precede the development of leukemia. Using the HRS/J mutant mouse they find that a defect in the thymus epithelium (hypo-maturation) leads to a decrease in Lyl positive T cells with subsequent expression of xenotropic virus and development of leukemia later in life. In the AKR leukemia model a hypermaturation of the epithelium leads to the development of Ia positive thymocytes expressing xenotropic GP70 and subsequently developing leukemia. Long term bone marrow cultures and chimeras using both of these models suggest the defect is in the epithelial signals to the bone marrow prothymocytes which are normal. The radiation leukemia model in C57Bl mice on the other hand have a preleukemic signal in the prothymocyte which is transformed during interaction with a normal thymic epithelium. The latter model shows depressed natural killer cell activity associated with the preleukemic state.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Marrow Transplantation

Principal Investigator: Dr. Paavo Toivanen
Performing Organization: Turku University
City and State: Turku, Finland

Contract Number: NO1-CB-74177
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Study the original and early differentiation of lymphoid stem cells in chickens.

Approach: Transplantation of stem cells or allogeneically educated, more mature cells into immunodeficient chicks or chicken embryos. Study the fate and function of transplanted cells by morphological and functional means. Determine whether stem cells originate in the yolk sac or intra-embryonically.

Progress: Studies on the role of embryonic bone marrow as a source of lymphoid stem cells have been continued by transplanting 12 day embryonic bone marrow into 12 day irradiated embryos. Due to a failure of electrocoagulation as a bursectomy procedures, a new modification for the early bursectomy (at 56-70 h of incubation) has been developed. Three-hundred operations have been carried out, and 3 successfully hatched chicks are waiting for functional and morphological analysis. Studies on the immediate antibody formation by bursa cells educated in an allogeneic bursal microenvironment have been completed. Allogeneically educated B cells have the same capacity for immediate antibody formation as syngenetically educated B cells. Studies on possible MHC-restriction after an allogeneic education of B cells have been completed. No evidence for a bursa-induced MHC-restriction was observed. Studies on the allogeneic education of prebursal stem cells have been continued. The results obtained are in agreement with those mentioned above. Studies on in vitro education of bursal stem cells on a cultured bursal epithelium have been continued. Studies with yolk sac-embryo chimeras to investigate yolk sac contribution (at 3-7 days of incubation) to the origin of lymphoid stem cells have been started.

Significance to Cancer Research: In order to understand disturbances in the immune surveillance system facilitating the development of malignancies, and to be able to manipulate the system therapeutically, detailed knowledge of its normal development is of fundamental importance.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cell Surface Immunobiology of Metastatic Tumors

Principal Investigator: Dr. Garth L. Nicolson
Performing Organization: University of California
City and State: Irvine, CA

Contract Number: NO1-CB-74153
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To study surface properties of tumor cells important in malignant tumor spread.

Approach: Use or derive rat and/or mouse variant cell lines with altered in vivo properties such as the ability to metastasize via lymphatics or blood to specific secondary sites.

Progress: The adhesive properties of lymphocyte-resistant lines have been examined; immune lymphocytes failed to bind during 30 to 60 minute incubations at 37° C. This assay has been performed in vivo by injection of highly metastatic cells into the circulation. After canulation of the heart, circulating tumor cells were removed at various times and their states of aggregation with lymphocytes and platelets examined. Metastatic tumor cells aggregate with these normal host cells during blood-borne transport before implantation occurs. Tumor-activated lymphocytes or their released lymphokines have been found to stimulate melanoma cell growth in vitro. Supernatants obtained from macrophages also stimulate melanoma cell growth in vitro. The contractor has examined in detail morphologic interactions of metastatic tumor cells with endothelial cell monolayers and endothelial extracellular matrix. Metastatic tumor cell destruction of the endothelial extracellular matrix is dramatically aided by the normal endothelial cells. Results indicate that tumor cells can induce the solubilization of the endothelial basement membrane-like matrix, but the degradation requires endothelial cells and may even be mediated by the endothelial cells stimulated by tumor cell interactions. Preliminary experiments indicate that ghost membrane preparations from metastatic human tumor cell lines can replace cells in stimulating endothelial cell turnover of extracellular matrix.

Significance to Cancer Research: A leading cause of death due to cancer is tumor metastasis, but little is known concerning the tumor and host properties important in metastatic tumor spread. Detailed information on the life-threatening, highly metastatic cell variants that exist in the primary tumor at low frequencies is necessary to develop suitable detection and therapeutic procedures to prevent metastasis.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Characterization of Antigen-binding T-cell Receptors

Principal Investigator: Dr. Joel W. Goodman
Performing Organization: University of California
City and State: San Francisco, CA

Contract Number: NO1-CB-74178
Starting Date: 9/16/77 Expiration Date: 9/15/80

Goal: Purify T cell-derived antigen-binding receptors; evaluate new procedures for detection of antigen-binding T cells.

Approach: Use the response of A/J mice to chemically defined antigen (p-azobenzenearsonate) for these studies.

Progress: Three T cell hybridomas from the fusion of azobenzenearsonate (ABA)-binding, affinity-purified A/J T cells with the AKR lymphoma cell line BW 5147 were studied for functional activity. These cell lines formed rosettes with ABA-sheep RBC which were inhibitable with soluble ABA-protein conjugates. Culture supernatants from these lines were tested for modulation of an *in vitro* primary anti-ABA response of A/J spleen cells to ABA conjugate of *Brucella abortus*. All three exerted a strong antigen-specific suppressor activity in this *in vitro* system. The suppressor activity was not significantly depleted by antigen immunoadsorbents, but was completely removed by an anti-I^k immunoadsorbent (A•TH anti-A•TL). The suppressor activity was unaffected by affinity chromatography using an immunoadsorbent prepared with monoclonal anti-I-A^k antibody. These findings indicate that the hybridomas secrete antigen-specific suppressor molecules which bear I-region coded determinants. These determinants are putatively I-J, although this has not yet been confirmed with a specific anti-I-J^k reagent. The suppressor molecules, while antigen-specific in functional assays, bind weakly to antigen matrices, suggesting a low affinity for the binding site.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Characterization of Antigen-binding T Cell Receptors

Principal Investigator: Dr. Frank W. Fitch
Performing Organization: University of Chicago
City and State: Chicago, IL

Contract Number: NO1-CB-74149
Starting Date: 9/19/77 Expiration Date: 9/29/80

Goal: Characterize antigen receptors on rat T cells which are specific for rat alloantigens.

Approach: Preparation of monospecific anti-idiotypic antisera; development of continuous cell lines of rat lymphoid cells which secrete homogeneous antibody reactive with rat alloantigens; screening of various rat T Cell populations for the presence of relevant idiotypic receptors.

Progress: Although misassembly of myeloma and antibody light and heavy chains is less extensive in rat-mouse hybridoma cells than in mouse-mouse hybridomas, enough misassembled molecules are produced to complicate both production of anti-idiotypic (anti-Id) antibody and interpretation of data obtained using anti-Id. Additional hybridomas producing Lewis anti-BN antibody reactive with Class I and Class II antigens have been produced using the non-secreting SP2/0 myeloma to avoid the problem of misassembled antibody molecules. One such hybridoma antibody (K.2.13), which causes renal allograft enhancement when administered together with donor lymphoid cells, has been used to raise rabbit anti-Id. This anti-Id blocks the binding of raiolabeled hybridoma to antigen-bearing erythrocytes but does not inhibit binding of other hybridomas. Rats have been immunized with hybridoma K.2.13 to produce rat anti-Id. Culture conditions for generating cytolytic T cells have been refined. Attempts to develop alloreactive T cell clones using conditions that have been highly successful with mouse lymphocytes have been unsuccessful, so far, using rat lymphocytes. Hybridoma antibodies reactive with rat lymphocyte subpopulations have been developed in order to characterize the subsets of cells with which anti-Id reacts. Conditions for optimal labeling of cell surface molecules and for polyacrylamide gel analysis of these molecules have been developed.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Reagents for Characterization of Human Cell Subpopulations

Principal Investigator: Dr. Robert Hunter
Performing Organization: University of Chicago
City and State: Chicago, IL

Contract Number: NO1-CB-84287
Starting Date: 9/30/78 Expiration Date: 9/29/80

Goal: Development of new reagents for the characterization of sub-populations of human cells important to the immune response or detection and characterization of new subpopulations of these cells.

Approach: Use lymphoid cells from mice immunized with human lymphoid cells in hybridization experiments to produce antibodies which react with human lymphocyte subclasses. Produce and characterize appropriate antibodies and determine their ability to define subsets of lymphocytes.

Progress: Progress in the last year includes a number of cell fusion experiments. A total of over two thousand clones have been screened using a microcytotoxicity assay and fifteen have been chosen for detailed study. These include a clone designated G4F8 which reacts with B but not T lymphoblastoid lines and appears to correlate with the presence of receptors for complement. A second clone, D3, appears to recognize an antigen found on blast cells but not resting normal lymphoid cells. A third, D2, appears to react with an antigen on a small proportion of thymic lymphocytes and most B and T cells in peripheral circulation. A number of avenues have been used in an attempt to increase the number of useful clones in a given fusion experiment.

Significance to Cancer Research: The development of reagents for characterizing subpopulations of human cells important to the human response may facilitate analyses and manipulation of the immune response to human tumors.

Project Officer: Ms. Judith Whalen
Program: Immunology Section
Tecnnical Review Group: Tumor Immunology Committee
FY 80 Funds:

CONTRACT RESEARCH SUMMARY

Title: Reagents for the Characterization of Human Cell Subpopulations

Principal Investigator: Dr. Marius Teodorescu
Performing Organization: University of Illinois
City and State: Chicago, IL

Contract Number: NO1-CB-84268
Starting Date: 9/30/78 Expiration Date: 9/29/80

Goal: Development of new reagents for the characterization of subpopulations of human cells important to the immune response or detection and characterization of new subpopulations of these cells.

Approach: Determine by in vitro tests the function in immune response of lymphocyte subpopulations isolated by specific absorption of human lymphocytes to a bacterial monolayer fixed on a glutaraldehyde-fixed gelatin bed.

Progress: The bacterial binding properties of various T cell subpopulations are being studied. Particular emphasis has been placed on subpopulations T_1 through T_4. A selection technique for the isolation of T_4 has been developed and the correlation of natural cytotoxic activity for T_4 has been found. It appears from other studies that T_2 is possibly involved in suppressive effects. Assays are being implemented to identify T helper cells in antibody formation.

Significance to Cancer Research: The development of reagents for characterization subpopulations of human cells important to the immune response may facilitate analyses and manipulation of the immune response to human tumors.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds:

CONTRACT RESEARCH SUMMARY

Title: Reagents for the Characterization of Human Cell Subpopulations

Principal Investigator: Dr. Noel Warner
Performing Organization: University of New Mexico
City and State: Albuquerque, NM

Contract Number: NO1-CB-84288
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: Development of new reagents for the characterization of subpopulations of human cells important to the immune response of detection and characterization of new subpopulations of these cells.

Approach: Develop specific heteroantisera against defined populations of human lymphoid cells stressing nonmalignant populations, including Tγ, Tμ and T null cells, and other T cell preparations, as well as B cell populations defined by various available cell surface markers, and macrophage subpopulations.

Progress: The general approach that has been developed in this project is to use flow cytometry to quantitatively analyze the expression of a variety of human lymphoid cell surface markers on normal and neoplastic cells, with the aim of then isolating distinct subpopulations from these preparations for their use as immunogens for developing new reagents. Although heteroantiserum production is continuing for certain areas, the contractor is now also developing procedures for the production of hybridoma derived monoclonal antibodies against selected cell surface components, both with mouse and human myeloma cell lines. The major rationale in this program has been to first identify which immunization schemes are potentially likely to yield antibodies of considerable interest for the definition of distinct subpopulations, and then to initiate hybridoma development in those instances. As a result, a large variety of reagents have been developed for identification and characterization of human mononuclear leukocyte subpopulations.

Significance to Cancer Research: The development of reagents for characterizing subpopulations of human cells important to the immune response may facilitate analyses and manipulation of the immune response to human tumors.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $97,713

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Susceptibility to Cancer

Principal Investigator: Dr. Geoffrey Haughton
Performing Organization: University of North Carolina
City and State: Chapel Hill, NC

Contract Number: NO1-CB-74182
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Investigate the finding that a polymorphic gene (or genes), linked to the H-2 complex in mice, determines susceptibility to primary sarcoma induction following neonatal injection of RSV-containing chicken tumor material.

Approach: Investigate the mechanism of action of the genes to determine whether differences in immune response to tumor antigens are involved. Explore the finding that both dominant and recessive resistance exist. Test for immune surveillance and immune stimulation.

Progress: The effect of immunologic manipulation on the development of primary murine Rous sarcoma was examined after neonatal injection of Rous sarcoma thymocyte serum (ATS) treatment of mice beginning at 7 and 21 days of age tripled and nearly doubled, respectively, the incidence of primary Rous sarcoma, but treatment at 21 days significantly prolonged the mean latency period of the resultant tumors. Lymphoid cells from syngeneic adult mice immunized with allogeneic Rous sarcomas (and resistant to syngeneic tumor challenge) had no effect when the animals received injections at birth (day 1), but immune lymph node cells (LNC) significantly increased tumor incidence when animals were given injections of LNC 1 week after birth and injections of RCTM on day 1. LNC and spleen cells (SC) from syngeneic adult mice that had been inoculated with RCTM at birth but had not developed tumors by 200 days of age (V^+T^-) decreased tumor incidence when administered on day 1 but not on day 7. Normal adult SC and LNC had no effect when given on day 1 but significantly increased tumor incidence when given on day 7. RCTM-inoculated mice were apparently susceptible only to immunologic manipulations that reduced primary tumor incidence during the first few days of life. After 7 days of age, all treatments had no net effect or increased tumor incidence, though ATS treatment beginning at day 21 significantly lengthened the mean tumor latency period.

Significance to Cancer Research: We have demonstrated as clear link between genetic control of tumor susceptibility and genetic control of tumor-specific immune responsiveness. Preliminary data suggest that different components of the immune response stimulate the growth of and eliminate tumors.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Selective Depletion of Mononuclear Phagocytes *In Vivo*

Principal Investigator: Dr. Stephen Russell
Performing Organization: University of North Carolina
City and State: Chapel Hill, NC

Contract Number: NO1-CB-84271
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: Development of methods for the selective *in vivo* destruction or sustained functional inactivation of mononuclear phagocytes.

Approach: Produce specific antimononuclear phagocyte globulin by selective absorption and assess the effect of these reagents on mononuclear phagocytes *in vivo* using "immune" granuloma formation in presensitized mice and spontaneous regression of Moloney Sarcomas in mice as model systems.

Progress: Rabbit antisera was raised against three sources of cells: cultured bone marrow mononuclear phagocytes, a macrophage cell line (RAW 264), and thioglycollate elicited peritoneal exudate cells. Preliminary *in vivo* screening demonstrated that the first of these was the most effective. Immunization protocols have been varied in several parameters in an effort to produce a maximally efficacious serum. It appears that this serum reacts with mononuclear phagocytes at most stages of differentiation and this is a desirable characteristic. Absorptions with murine RBC and mastocytoma P815 have been used to eliminate most unwanted cross reactivities but further absorption with purified neutrophils seems to be required. IgG is the most active of the immunoglobulin fractions yet tested for activity and when administered intravenously is capable of depleting mononuclear phagocytes for a period of approximately twenty-four hours.

Significance to Cancer Research: Studies on the *in vivo* depletion of macrophages will aid in examining the roles of macrophages in tumor destruction.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $78,689

CONTRACT RESEARCH SUMMARY

Title: Immunogenicity of "Spontaneous" Animal Tumors

Principal Investigator: Dr. Michael J. Embleton
Performing Organization: University of Nottingham
City and State: Nottingham, England

Contract Number: NO1-CB-64009
Starting Date: 6/18/76 Expiration Date: 10/14/79

Goal: To determine (a) the frequency of expression of tumor-associated antigens capable of inducing host resistance in spontaneously arising tumors of an inbred rat strain, and (b) the influence of these antigens on tumor development.

Approach: Spontaneous rat tumors arising in a normal breeding population are transplanted into syngeneic hosts and their immunogenicity is assessed by immunization/challenge protection studies in vivo, carried out mainly during early transplant generations. The transplantation studies are supported by serological testing for antibodies reactive against tumor cell surface antigens.

Progress: A total of 41 spontaneous WAB/Not rat tumors have been tested for immunogenicity in syngeneic hosts. Various immunization and challenge procedures were tried and the methods adopted as standard were implantation of irradiated (15,000R) tumor grafts or surgical resection of subcutaneously growing tumor, followed by subcutaneous challenge with trypsinized tumor cell suspensions. Immunity was induced against 7 tumors. In 4 cases the response was very weak, but three tumors showed immunogenicity such that immunized rate rejected approximately 100 times the tumor inoculum growing in controls. In the case of one of these tumors immunogenicity was lost on prolonged passage in vivo, but the other two (mammary carcinoma Sp4 and fibrosarcoma Sp7) were consistently immunogenic. Antigens on these tumors were not shared by histologically similar tumors as judged by transplantation tests. Tumor Sp4 induced good antibody responses in syngeneic rats, but other tumors were less consistent in this respect. Tests for cell-mediated cytotoxicity revealed only natural killer (NK) cell activity in tumor bearing animals, with no tumor-specific component. It was concluded that only a minority of spontaneous rat tumors are immunogenic, and that immunologic manipulations offer little hope for tumor therapy or detection at the present time.

Significance to Cancer Research: Spontaneous tumors are more relevant to human tumors than are experimentally induced tumors. These studies therefore suggest that some human tumors could have specific antigens relevant for host immunity.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Detection and Development of H-2 Recombinant and Mutant Strains

Principal Investigator: Dr. Donald C. Shreffler
Performing Organization: Washington University
City and State: St. Louis, MO

Contract Number: NO1-CB-74154
Starting Date: 3/1/77 Expiration Date: 2/28/80

Goal: The detection and fixation in congenic inbred strains of new recombinant and mutant H-2 haplotypes, particularly variants involving the I (immune response) region of the H-2 gene complex.

Approach: Screen large numbers of progeny from crosses involving different H-2 haplotypes for: (1) testing of mutagens that may enhance the frequencies of recombination and mutation; (2) screening of progeny of newly arisen recombinants for second-step recombination events; and (3) development of MLR and Ia serotying methods for detection fo I region recombinants and mutants.

Progress: Over the final contract year some 4,400 progeny from 17 different crosses were screened and 21 putative new intra-H-2 recombinants were detected. Of those that have been proven by progeny test so far, 11 are being carried as new congenic lines and 8 are still undergoing testing. By screening 520 progeny of mutagen-treated heterozygous males, three possible recombinants have been found and are being progeny tested. Mutagen treatment seems to enhance intra-H-2 recombination, but there may be some instability in the newly recombinant chromosomes. A pilot test of MLR screening methodology has been started and among 100 heterozygous progeny screened, three have been found to significantly stimulate a presumed syngeneic test cell. Progeny tests are under way and it remains unclear whether these are true genetic variants. An improved method of screening for I region variants may permit efficient detection of both recombinants and mutations among the same progeny.

Significance to Cancer Research: The H-2 complex plays a critical role in susceptibility to oncogenic viruses. The new strains produced will aid in the definition of the genes that control susceptibility. The products of K, D, and I regions of the complex are involved in cell-mediated reactions against neoplastic cells. These new strains will provide material for characterization of these products.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Molecular Studies of Specific Cell-mediated Cytotoxicity

Principal Investigator: Dr. Gideon Berke
Performing Organization: Weizmann Institute
City and State: Rehovot, Israel

Contract Number: NO1-CB-74183
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Elucidation of the mechanism whereby cytotoxic T lymphocytes (CTL) interact with and kill target tumor cells (TC) in vitro.

Approach: Isolate and characterize a cell-free, functionally reactive component of CTL and study its mode of action on the target cells; determine the role of membrane lipid composition and microviscosity on CTL-TC interaction; do biochemical studies on CTL-TC contact regions; evaluate the possibility that TC has an active role in the lytic process.

Progress: The group has confirmed that CTL-blasts, generated in vitro or obtained from 'educated' spleens in vivo, possess high levels of a membrane glycoprotein (ca. 145,000 daltons - T145). However, they have shown that T145 was absent on CTL induced in vivo by a standard intraperitoneal alloimmunization protocol. Furthermore, no correlation has been found between cytolytic activity and expression of T145 on various CTL populations. Proteolytic digestion of CTL, which fully inhibits their lytic activity, did not reduce the level of T145 labeling as determined by surface iodination indicating that this glycoprotein might not be involved in lysis. Therefore, the suggestions that T145 is a unique surface marker present on all CTL and is involved in lysis should be reconsidered. The finding that several CTL populations are consistently T145 positive while others are T145 negative may be due to active CTL in two stages of differentiation or may represent different CTL classes or subpopulations. On the other hand, evidence has been presented to implicate involvement of a CTL-surface component of 23,000 daltons in CTL-TC interaction. The group has examined whether CTL projections penetrating TC play an obligatory role in the lytic process or merely function in bridging effector and TC. These and other results suggest that the CTL projects probably play a role only in CTL-TC binding and are necessary, but not sufficient, for the induction of lysis.

Significance to Cancer Research: The precise understanding of the interaction of lymphocytes and tumor cells could (1) clarify the role of the immune system in the response against tumors, (2) provide reason(s) for its failure in the tumor-bearing patients, and (3) contribute to a more rational approach to immunotherapy of tumors.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Induction of Functional Differentiation of T Cells

Principal Investigator: Dr. Martin Haas
Performing Organization: Weizmann Institute
City and State: Rehovot, Israel

Contract Number: NO1-CB-74180
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To establish methods for in vitro leukemogenesis of lymphocytes on thymus reticuloepithelial cells through analysis of the role of retroviruses, identification of target lymphocytes, and purification of different types of thymus reticuloepithelial cells.

Approach: Establish culture conditions for growing reticuloepithelial cell monolayers from leukemic and normal mice. Monolayer cultures will be analyzed for cell type, retrovirus production, and sensitivity to infection. The reticuloepithelial cell monolayers will be used in cocultivation experiments with lymphocytes in order to study differentiation of different types of lymphocytes and their transformation into leukemic cells.

Progress: Extensive experimentation on the development of bone marrow colonies in soft agar plated on feeder layers derived from thymus, bone marrow or embryo cells has shown that no feeder activity increased the relative number of lymphoid colonies. Crude thymus cell growth factor was not effective either. Thus the previously observed blastogenic response of thymus or bone marrow cells cultivated on thymus epithelial cells is of short duration and measurable only upon inoculation of these blast cells into mice, but not in vitro. Moreover, the blastogenic effect of thymus epithelial cells could be replaced by mitogens. Retroviruses that were isolated from x-ray induced lymphomas of B6 mice have been compared by "serologic mapping" of the virus-associated cell surface antigens and, in part, by tryptic digest mapping of viral p30 and gp70. T cell lymphoma-inducing and B cell lymphoma-inducing virus isolates were shown to possess different gp70 molecules, probably representing different recombination within the gene coding for the envelope glycoprotein. Moreover, within this single mouse strain a large number of different recombinant viruses were found, only few of which induced either BCL or TCL.

Significance to Cancer Research: In vitro induction of lymphosarcomas, isolation, and analysis of the causative agents could lead to an understanding of the induction of spontaneous lymphosarcomas.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Susceptibility to Tumors

Principal Investigator: Dr. Nechama Haran-Ghera
Performing Organization: Weizmann Institute
City and State: Rehovot, Israel

Contract Number: NO1-CB-74151
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To study genetic control of susceptibility to tumors.

Approach: Investigate the number, genetic map position, mutual relationship, and function of H-2 linked resistance genes to murine leukemia, including a study of genetic regulation of the phases in leukemogenesis.

Progress: The genetics of H-2 linked resistance to four RadLV variants has been investigated. In leukemogenesis induced by two of these (RadLV and RS-RadLV), I region and H-2D linked loci, whereas in another two (A-RadLV and D-RadLV) only I region linked loci are involved in resistance. These localizations are consistent with the involvement of Ir genes in resistance to murine leukemia. Other experiments show that only mice of the resistant H-2 haplotype developed transplantation resistance, which could be transferred to naive recipients, by lymphoid cells, strongly supporting this role of Ir gene control in resistance to virus induced murine leukemia. Resistance of virus infected resistant x sensitive F1 mice to parental lymphoma cells was found to be H-2 restricted, namely only tumors of the resistant parental haplotyope were rejected. This finding suggests that the immunogen is presented in an H-2 associated, i.e. most likely cell bound form. A pathway of leukemia pathogenesis could be described which includes: 1. infection, 2. appearance of preleukemic cells, 3. their immunological arrest in the H-2 linked resistant genotype, or their proliferation in the H-2 linked sensitive genotype, 4. growth of fully transformed cells and clinical disease in the "non-arrested" H-1 linked sensitive, i.e. "low responder" strains.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunobiology of Metastases

Principal Investigator: Dr. Shraga Segal
Performing Organization: Weizmann Institute
City and State: Rehovot, Israel

Contract Number: NO1-CB-74185
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Further analysis of the antigenic diversity of lung metastases generated by the 3LL tumor.

Approach: Generation of cytotoxic T-lymphocytes in culture against M-3LL and in L-3LL and cross-testing their cytotoxic effects.

Progress: The T-10 sarcoma induced by methylcholanthrene in (C3H x C57BL/6)F1 mice, transplanted subcutaneously or intra foot pad into syngeneic mice, metastasizes in the lungs. Several T-10 derived clones, but no all of them, generated lung metastases when injected intravenously into immunologically intact mice. In spite of the tumor's hybrid origin ($H\text{-}2^k$ x $H\text{-}2^b$), only a few of the clones expressed both parental haplotypes. As a rule, only the clones which expressed both parental haplotypes formed lung metastases. The clones not expressing the $H\text{-}2^k$ haplotype were incapable of generating lung metastases. The metastatogenic capacity of the T-10 clones correlated well with their ability to express parental H-2 haplotypes, yet this capacity was not entirely dependent on their differential homing affinity to the lungs. Even clones which had failed to generate metastases in immunologically intact animals did form lung metastases in recipients which were immunologically suppressed by sublethal irradiation. These results strengthen our previous findings indicating diversity among tumor cells generating metastases and their primary ancestor. Furthermore, the ability to disseminate and form metastases seems to be a well controlled intrinsic property of individual tumor cell subpopulations comprising a given tumor. In some tumors, such as the T-10 sarcoma, it is possible that the ability of these subpopulations to colonize a particular organ is not solely dependent on their homing characteristics; to a large extent, it may be influenced by their ability to resist the host's immune system which may prevent their dissemination through blood and lymphatic vessels.

Significance to Cancer Research: The fact that metastases differ antigenically from their primary tumor ancestry may contribute to our understanding of mechanisms by which metastases avoid immune destruction by the host. In addition, these findings may lead to more rational approaches to the problem of immunotherapy and control of metastasis spread and growth.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Production and Detection of Antibodies to Chemical Carcinogens

Principal Investigator: Dr. Helen Van Vunakis
Performing Organization: Brandeis University
City and State: Waltham, MA

Contract Number: NO1-CP-23243
Starting Date: 4/20/72 Expiration Date: 1/31/80

Goal: To develop immunoassays that can be used to determine carcinogens, procarcinogens, cocarcinogens, and their metabolites in physiological fluids, tissue extracts, and other material. Such assays also facilitate studies on pharmacokinetics and metabolic conversions, and are useful in the characterization of enzyme systems.

Approach: The compound is coupled to a macromolecular carrier that promotes its antigenicity and the resulting conjugate is used for immunization of animals. Resulting antibodies are used to devise radioimmunoassays.

Progress: During the current contract period an assay for N-nitrosonornicotine (NNN) has been developed and used to follow the disposition of NNN in the physiological fluids of rabbits and examine the sera of smokers. The contractor has obtained antibodies specific for nornicotine. Analysis of human saliva and sera shows that saliva can be used to determine smoking status. In vitro experiments have shown that nicotine and cotinine analogues of nicotinamide dinucleotide can inhibit certain dehydrogenases. Most recently the contractor has analyzed tissue samples of two smokers and three nonsmokers for nicotine and cotinine analogues of nicotinamide mononucleotide and nicotinamide dinucleotide. The analogues were only detected in the smokers' tissue samples.

Significance to Cancer Research: In addition to monitoring levels of noxious agents in the environment, the availability of such assays facilitates basic studies on carcinogens, procarcinogens, and cocarcinogens that may help us understand how they exert their biological activities.

Project Officers: Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Induction of Colonic Tumors in Guinea Pigs

Principal Investigator: Dr. Gary L. Cockerell
Performing Organization: Cornell University
City and State: Ithaca, NY

Contract Number: NO1-CP-65744
Starting Date: 9/30/76 Expiration Date: 9/29/80

Goal: To produce carcinomas of the colon in approximately 700 NIH guinea pigs and subsequently test the immunotherapeutic effect of BCG vaccines versus other modalities of treatment.

Approach: N-methyl-nitrosourea is administered by intrarectal installation to produce colonic carcinomas. Following tumor induction, laparotomies are performed, and the tumorous portion of bowel is (1) injected intralesionally with BCG vaccines, (2) surgically resected and a diverting colostomy established, or (3) located but not otherwise treated. Animals are then allowed to recover from surgery and are observed until death.

Progress: Two sets of 240 guinea pigs each were exposed to the intrarectal administration of methylnitrosourea (MNU) for 14 to 35 weeks to induce colonic adenocarcinomas. At 24, 28 or 35 weeks post start of MNU administration, groups of guinea pigs were treated either by intratumoral injection of BCG or surgical excision of tumors and compared to non-treated animals. Most guinea pigs died within one year of therapy and there was no difference in survival rate between treated and non-treated groups. The leukocyte migration inhibition assay, lymphocyte blast transformation assay with variations reaction, and macrophage cytotoxicity assays have been used to evaluate the immune reactivity of MNU-exposed guinea pigs. Macrophage cytotoxicity has been the only assay to yield useable results but there are yet insufficient therapeutic modalities. A third set of 240 guinea pigs has been exposed to lesser amounts of MNU to derive carcinogen dose response data. These data will make it possible to examine the effects of immunotherapy of surgical treatment on tumors which arise in a smaller percent of MNU-exposed animals and which might therefore be less malignant.

Significance to Cancer Research: The guinea pig colon carcinoma model will serve as a useful system to test various methods of tumor treatment, since it has now been shown that the guinea pig colon tolerates intramural BCG injection and is surgically resectable.

Project Officers: Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Antigenicity of Precancerous Lesions in Animal Models

Principal Investigator: Dr. Richard G. Olsen
Performing Organization: Ohio State University
City and State: Columbus, OH

Contract Number: NO1-CB-74159
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To determine whether precancerous cells carry antigenic markers which are distinguishable either in type or concentration from those exhibited by normal or by malignant cells.

Approach: Using the Rickard strain of feline leukemia virus (FeLV) in SPF cats, study will be made of the sequential development of feline oncornavirus associated cell membrane antigen (FOCMA) in preleukemic bone marrow cells in cats infected with FeLV. Correlations will be sought with other markers associated with the preleukemic phase of the disease.

Progress: Bone marrow FOCMA was induced early (2-4 weeks p.i.) in both groups of cats challenged with R-FeLV but attained a higher level in cats which became gsa positive. B.M. FOCMA in individual cats appeared to wax and wane in cycles. Even in gsa negative cats after soluble gsa antigen was no longer detectable, FOCMA continued to appear and disappear in both B.M. and serum. This characteristic of FOCMA was also found in gsa positive cats which were also found to have cyclic but always high levels of serum gsa. Antibody to virus and to FOCMA cells and virus neutralizing antibody was significantly higher in gsa negative than in gsa positive cats. Only viremic cats were lymphopenic, and only viremic cats had a sustained decrease in suppressor cell function. Gsa negative cats had an initial decrease but recovered suppressor cell activity. Preliminary results suggest that leukemic cells may not have significantly more FOCMA than preleukemic B.M. cells.

Significance to Cancer Research: The immunologic rejection of tumor tissue is recognized as the pivotal factor in the control of cancer. These studies will determine whether qualitative and/or quantitative differences in tumor antigen expression on the early preneoplastic lesion influences the subsequent course of tumor development.

Project Officer: Ms. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunoprevention of Tumors in Rabbits

Principal Investigator: Dr. John W. Kreider
Performing Organization: Pennsylvania State University
City and State: Hershey, PA

Contract Number: NO1-CB-74158
Starting Date: 9/29/77 Expiration Date: 9/28/80

Goal: To develop immunologic maneuvers for interfering with the progression of solid tumors from the premalignant to the malignant stage.

Approach: Using the Shope papilloma in the rabbit, a naturally occurring model of neoplastic progression, attempts will be made to intervene in the progression from the preneoplastic stage using nonspecific (BCG, C. parvum) and specific (tumor cell vaccines) immune stimulants.

Progress: Three different approaches have been used in attempt to intervene in the progression of the Shope papilloma-carcinoma complex: (1) Treatment of papillomas with an allogeneic papilloma cell vaccine given alone or in combination with various doses of BCG; or (2) treatment of papillomas with intratumoral injections of various immune stimulants (BCG cell walls on oil droplets, C. parvum, DNCB or adriamycin); or (3) direct injection of early papilloma-derived epidermoid carcinomas with immune stimulants (CWOD). The growth rates of papillomas, and the frequency of carcinomatous transformations have been followed. Since carcinomas may not appear for 1-2 years, long-term follow-up observations on carcinomas are necessary. The cell vaccine, alone and combination with 10^7 CFU significantly reduced the incidence of 10^8 or 10^6 CFU. Treatment of early primary carcinomas in this experiment with i.t. CWOD did not alter tumor growth rates or host survival. In the second experiment, i.t. injections of 0.5 or 1.0 mg CWOD beginning on day 22 and at 1-2 month intervals thereafter did not affect tumor growth. In the third experiment, treatment of small early papillomas was begun at weekly (for 5 weeks) and at 7 week intervals using a variety of agents. DNCB paintings significantly stimulated papilloma growth and were associated with a reduced regression frequency. This result may be due to direct stimulation of papilloma cells. Adriamycin destroyed papillomas and adjacent skin through direct toxicity. CWOD significantly reduced papilloma growth rates and was associated with an increased regression frequency. C. parvum increased the incidence of regression and similarly slowed papillomas growth rates. Tumor rejection immunity has been tested by challenging rabbits with skin autografts infected with SPV while in residence on nude mice for a few days. Preliminary analysis indicates that regressor rabbits usually suppress the growth of these secondary papillomas and carcinomatous rabbits often support luxuriant growth.

Project Officer: Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cytotoxic Activity of Syngeneic Complement

Principal Investigator: Dr. Leon Rosenberg
Performing Organization: Stanford University
City and State: Stanford, CA

Contract Number: NO1-CB-84245
Starting Date: 9/30/78 Expiration Date: 9/29/80

Goal: Study the interactions of tumor cells, anti-tumor cell antibody, and complement in completely autologous or syngeneic systems comparing normal, premalignant, and malignant cells.

Approach: Identify, characterize, and attempt neutralization of a proposed membrane-associated factor responsible for blocking activity of syngeneic complement against tumor target cells.

Progress: Agglutinating antibody against C. parvum, the agent which cross reacts with the membrane associated anti-complementary factor, has been raised in 15 guinea pigs in the course of the experimenmts designed to test the hypothesis that cells from guinea pigs tested with C. parvum will be more susceptible to lysis by syngeneic complement. It is proposed that the antibody might neutralize the anti-complementary factor. The contractor plans to look for the putative autoantibody against the membrane-associated factor with an anti-idiotypic antibody. The agglutinated C. parvum has been saved for injection into guinea pigs for the purpose of attempting to raise such an anti-idiotypic antibody; this antisera should be ready for testing within a month or two.

Project Officer: Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunologic Mechanisms of Cattle

Principal Investigator: Dr. Charles Muscoplat
Performing Organization: University of Minnesota
City and State: St. Paul, MN

Contract Number: NO1-CB-84244
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: Perform in vitro studies of immunologic mechanisms in cattle in conjunction with ongoing immunoprophylaxis studies to gain information on the possible influence of immunity on carcinogenesis.

Approach: Perform in vitro studies on the cellular and humoral events of the bovine immune system on tissues provided by NCI from experimental animals involved in a study of immunoprophylaxis of bovine ocular squamous cell carcinoma to determine whether correlative changes in immune response can be detected during the process of cancer development and, if so, to determine the immunologic mechanisms involved.

Progress: The contractor has been able to initiate and successfully maintain tumor cells in tissue culture from a very high percentage of tumors, i.e. 90%. Also the ability to obtain and to maintain the normal cells in culture is at very high percentage. A mixed antiglobulin reaction assay has been used to determine the presence of surface antigens on cultured cells of benign ocular tumors from cattle with ocular squamous cell carcinoma. Antibody-dependent cell-mediated cytotoxicity was conducted on cultured cells obtained from precurser lesions of squamous cell carcinoma. In several experiments, it was found that autologous serum enhances the killing of tumor cells with non-immune lymphocytes; however, no killing was observed with normal serum. An indirect immunofluorescence test was used to detect surface antigen on tumor cells. A pattern of fluorescence was observed which suggests that the tumor specific antigen is present on the cell surface. No fluorescence was observed when normal serum was used. At present, there are 14 cell lines from precursor lesions, 4 cells lines from animals who have progressed to papilloma and 4 cell lines obtained from carcinoma. Also, there are 6 cell lines obtained from normal corneal cells. All these cells are either in culture or stored in liquid nitrogen for later use. Serum obtained from these animals with different stages of disease and from normal animals are frozen for later use.

Project Officer: Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $87,800

CONTRACT RESEARCH SUMMARY

Title: Immunoprophylaxis of Bovine Lymphosarcoma

Principal Investigator: Dr. Richard M. Thorn
Performing Organization: University of Pennsylvania
City and State: Philadelphia, PA

Contract Number: NO1-CB-84243
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: Study the immunoprophylaxis of bovine lymphosarcoma through administration of BCG cell wall vaccine by intravenous injection as a possible guide for the control of analogous human disease.

Approach: Study the effect of the administration of BCG cell wall vaccine on the extent and rate of development of lymphosarcoma in cattle injected with bovine leukemia virus at a stage prior to the development of discernible evidence of disease.

Progress: Animals which had received 3 mg of BCG cell wall skeleton (BCG-CWS) 6/5/79 were re-inoculated with 30 mg intravenously 10/23/79. In spite of this second exposure, the contractor found no in vitro evidence of sensitization to BCG antigens. All animals in this experiment were BLV infected at the time of the second injection. Five BCG and 3 control cows also had enlarged, firm lymph nodes and other clinical signs suggestive of lymphosarcoma, but this was not confirmed in 5 of these animals at the next physical. BSA gradient separations of lymphocytes from 5 pairs of cows 3 months after the first BCG treatment were done. The majority of cells were always in fractions 5 and 6, but the proportion of EAC rosette-forming and syncytia-forming cells was uniform for all fractions. The spontaneous thymidine incorporating cells followed almost the same pattern as cell recovery. These patterns were also observed before treatment and therefore, 3 mg of BCG-CWS had no effect on the distributions of these subpopulations. It is not yet known whether the second 30 mg injection altered their activity.

Project Officer: Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $160,012

CONTRACT RESEARCH SUMMARY

Title: Immunoprophylaxis of Cancer Eye in Cattle

Principal Investigator: Dr. Stephen J. Kleinschuster
Performing Organization: Utah State University
City and State: Logan, UT

Contract Number: NO1-CB-74155
Starting Date: 9/16/77 Expiration Date: 9/15/80

Goal: The goal of this research is to develop immunologic maneuvers for interfering with the progression of solid tumors from the premalignant (benign) to the malignant state and to relate the immunologic status of experimental animals to clinical events.

Approach: Intralesional administration will be made of BCG cell wall vaccine into benign ocular lesions of cattle to determine whether such treatment will retard or prevent the normal progression of these lesions to the malignant state. Additionally, the immunologic status of animals will be monitored via PPD skin testing in an attempt to correlate immunocompetence with regression, arrest, or progressive disease.

Progress: To date, 240 tumor bearing animals have been acquired, biopsied, ear tagged, photographed, skin tested with PPD, and randomized into the experimental groups which are: (1) untreated control, (2) sham vaccine, (3) local surgery, (4) active vaccine. All animals have received their experimental treatment and clinical events are being monitored along with PPD skin testing in order to correlate prognoses with immunocompetence. Results to date indicate that intralesional injection of BCG is effective in reducing the probability that progression to malignancy will occur. Additionally, the contractor is also collecting and cryopreserving blood components and tissue from each animal on a regular basis in order to provide material for Dr. Charles Muscoplat for in vitro studies regarding immunologic variables concomitant with change in clinical status.

Significance to Cancer Research: Studies involving the immunologic interruption of the transformation of a benign precursor lesion to one of malignancy at the earliest possible stage provide valuable contributions to the overall cancer effort. Additionally, via a program of monitoring the immunocompetence of experimental animals as related to clinical events following experimentation, relevant information can be contributed to the establishment of effective immunologic testing needed in order to evaluate therapy and provide guidelines for improved human clinical protocols.

Project Officer: Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds:

CONTRACT RESEARCH SUMMARY

Title: Intrapleural BCG After Primary Surgery for Lung Cancer

Principal Investigator: Dr. Martin F. McKneally
Performing Organization: Albany Medical College
City and State: Albany, NY

Contract Number: NO1-CB-53940
Starting Date: 6/1/75 Expiration Date: 9-30-81

Goal: To evaluate the therapeutic efficacy of intrapleural BCG in patients with lung cancer.

Approach: Following surgical resection of their tumors, patients with lung cancer had been randomly assigned to receive intrapleural BCG plus INH or INH alone. Subsequently, the design was altered so that test Stage I patients now receive BCG plus INH followed by additional supplemental cutaneous doses of BCG, while control patients received the BCG plus INH but no supplement.

Progress: 50 patients with Stage I lung cancer have entered the current randomized study (postoperative intraplueral BCG and oral INH with or without repeated cutaneous injections of BCG). There have been no recurrences and no deaths among the 21 patients in the group which receives cutaneous BCG, while 5 patients of 29 in the single intrapleural dose BCG have developed recurrent lung cancer; 2 have developed new primary cancers; and 1 has died from causes unrelated to malignancy. The differences between these two groups does not reach the level of statistical significance, $p=.1$.

Stage I patients treated with postoperative single dose intrapleural BCG and INH in the original study continue to demonstrate a lower recurrence rate than patients in the control group (surgery plus INH), $p=0.003$.

Significance to Cancer Research: The original randomized controlled trial shows statistically significant treatment effect due to immunotherapy. A repeat trial with larger numbers of patients is being conducted to try to confirm the result.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $129,768

CONTRACT RESEARCH SUMMARY

Title: Long-term Tumor-specific Cytotoxic Lymphocytes in Treatment of Mouse Leukemia

Principal Investigator: Dr. Kendall A. Smith
Performing Organization: Dartmouth Medical School
City and State: Hanover, NH

Contract Number: NO1-CB-74141
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To evaluate the usefulness of tumor-specific cytotoxic lymphocytes maintained in continuous culture for the prevention and treatment of mouse leukemia.

Approach: Attempts will be made to (1) demonstrate the tumor-antigen reactivity of human and murine cytotoxic T-cell lines; and (2) test in vivo the immunoprophylactic and immunotherapeutic value of murine cytotoxic T cells maintained in continuous proliferative culture(CTLL).

Progress: Preliminary experiments show that murine CTLL (1×10^7 cells) may be effective in prolonging survival when administered three days after a lethal IV dose (1×10^3 cells) of leukemic cells. Progress was made toward the construction of a murine model to test the use of CTLL for the eradication of leukemia cells prior to autologous bone marrow infusion in mice which received lethal irradiation.

The investigators report that the supplementation of cultures comprised of normal unprimed murine spleen cells and syngeneic tumor cells with partially purified, lectin-free TCGF leads to the generation of a primary in vitro cytotoxic response.

Based on his data, the investigator has constructed a model which depicts the biological role of TCGF in the T-cell immune response.

Significance to Cancer Research: The ability to cultivate large quantities of tumor-specific cytotoxic lymphocytes which retard in vivo tumor growth may provide a new approach to cancer treatment. The successful culture of differentiated functional T cells for extended periods offers the promise of new understanding of the mechanisms of cellular immunity and of immunodeficient and hyperimmune disease states.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds:

CONTRACT RESEARCH SUMMARY

Title: Studies of Immune Stimulants in Patients Receiving Radiation Therapy

Principal Investigator: Dr. John R. McLaren
Performing Organization: Emory University
City and State: Atlanta, GA

Contract Number: NO1-CB-64061
Starting Date: 7/16/76 Expiration Date: 6/29/80

Goal: To determine whether radiation therapy modifies the immunocompetence of patients with lung, head and neck, or cervical malignancies and to determine whether aggressive immunotherapy with combined BCG and levamisole may alter such modifications in immunocompetence and correlate with disease-free intervals or survival times.

Approach: T- and B-lymphocyte levels and percentages, in vitro lymphocyte transformation by mitogen, and skin test determinations are made on cancer patients at appropriate intervals before and following radiation therapy, and on a control population. Following radiation therapy, patients are randomized into arms with or without immunotherapy. Clinical, laboratory, X-ray, and immunological evaluations will be continued for sufficient intervals to observe disease-free intervals and survival times.

Progress Report: A total of 242 patients have entered this study divided among three histological site groups as follows: 1) head and neck cancer, 79 patients 2) lung cancer, 92 patients and 3)cervical cancer, 71 patients. Of these 34% have expired, an additional 16% have developed metastasis or recurrences, and 13% have refused treatment or have been lost to follow up. Studies are underway to evaluate T & B cell quantitation, mitogen assays and skin tests and to attempt to correlate these immunological parameters with clinical outcome and type of therapy received (immunotherapy or no immunotherapy).

Significance to Cancer Research: The data corroborate mounting evidence that cancer patients have suppressed immunocompetence, and that radiation further depresses many of these immune parameters. It is important to learn the significance of depression upon relapse and survival times.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy in Outbred Cat Lymphomas and Leukemias

Principal Investigator: Dr. Myron Essex
Performing Organization: Harvard College
City and State: Cambridge, MA

Contract Number: NO1-CB-64001
Starting Date: 6/1/76 Expiration Date: 5-31-81

Goal: To see if immunotherapy using antibody to a specific tumor antigen sustain clinical remission in outbred cats with naturally occurring thymic lymphoma.

Approach: Cats with chemotherapy-induced complete remission of thymic lymphoma are randomly assigned to no further therapy or treatment with antiserum against feline oncornavirus associated cell membrane antigen (FOCMA). Animals are monitored clinically and immunologically.

Progress: Twenty-two cats have been entered into the protocol. Among these there was 1 early death, 5 that failed to enter complete remission and 16 that achieved complete remission. A few cats had to be eliminated because of early deaths, but 13 were evaluated after randomization. While all 5 cats receiving no serum have relapsed, 3 of 8 that received serum are still in remission. Furthermore, 4 of 5 (80%) that received no serum have relapsed within 6 weeks after cessation of chemotherapy, while only 2 of 8 (20%) that received serum had relapsed within the same period. In one cat serum injection resulted in anaphylaxis which responded to epinephrine and oxygen.

The levels of serum FOCMA antibody titers were low in all cats, despite the fact that they received high titered serum. All cats were serially tested for the presence of FeLV. A few became either transiently or permanently nonviremic as a result of immunotherapy, but this did not have any obvious effect on clinical course. Sera from many FeLV exposed immune cats, which contained high FOCMA titers, were assayed after heat inactivation for antibody dependent cell cytoxicity (ADCC) activity using ^{51}Cr labeled cat tumor cells. The results have been negative despite a variety of effector cell donors and assay conditions.

Significance to Cancer Research: The cat system is probably the only model where studies with spontaneous lymphoid tumors are both economically and scientifically feasible because sufficient knowledge of the tumor antigen-immune system has been developed.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $64,970

CONTRACT RESEARCH SUMMARY

Title: Adjuvant Tumor-specific Active Immunotherapy of Squamous Cell Carcinoma of the Lung

Principal Investigator: Dr. Hiroshi Takita
Performing Organization: Health Research, Inc.
City and State: Buffalo, NY

Contract Number: NO1-CB-64007
Starting Date: 6/16/76 Expiration Date: 6-15-81

Goal: To evaluate the therapeutic effects of immunotherapy with tumor vaccine given adjuvant to curative surgical resection in squamous cell carcinoma of the lung by a prospective randomized study.

Approach: Following lung resection, patients with Stage I and II squamous cell lung carcinoma are randomized into 3 groups: Group I--control(no treatment; Group 2--specific immunotherapy; and Group 3--nonspecific immunotherapy. The specific immunotherapy group will receive solubilized squamous cell lung carcinoma antigen (500 mcg) with complete Freund's adjuvant. The non-specific immunotherapy group will receive Freund's adjuvant only.

Progress: Eighty patients have entered the study. Of this total, eight have been disqualified. Twenty-seven patients entered the control group (no immunotherapy); 21 patients received lung carcinoma associated antigen and complete Freund's adjuvant; and 24 patients received complete Freund's adjuvant. An interim analysis shows a statistically significant improvement in disease free interval and survival for each of the immunotherapy groups when compared with the control group.

Serial skin tests with DNCB, PPD and tumor associated antigen have not proven to be of prognostic value.

Significance to Cancer Research: The interim results of our study indicate that adjuvant tumor-specific immunotherapy may be effective. Immunologic monitoring of the patients reveals possible effective sensitization of the immunized patients to the tumor antigen.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $54,469

CONTRACT RESEARCH SUMMARY

Title: In Vitro Immunization with Autochthonous Human Tumor

Principal Investigator: Dr. Eva Klein
Performing Organization: Karolinska Institute
City and State: Stockholm, Sweden

Contract Number: NO1-CB-74144
Starting Date: 9/30/77 Expiration Date: 9-29-81

Goal: To demonstrate the existence of specific human tumor-associated antigens and to show in vitro increased responses to such antigens after in vitro immunization with autochthonous tumor.

Approach: Human tumor specimens (carcinoma of the lung, astrocytomas, osteo- and soft tissue sarcomas) will be characterized and fractionated for tumor and lymphoid cells. Cytotoxicity tests will be performed with biopsy target cells using blood lymphocytes and tumor infiltrating lymphocytes, as effectors, both directly at harvest and after cocultivation in vitro. Attempts will be made to characterize the nature of the effector cell.

Progress: The investigator has studied the autologous response to human tumor biopsy cells in two in vitro tests: (1) the autologous tumor stimulation (ATS) and (2) autologous lymphocytotoxicity (ALC). Over the last year, emphasis has focused on the latter.

ALC was positive in 28% of the 198 cases tested (mainly osteosarcomas and lung carcinomas). The tumor biopsy cells were only exceptionally sensitive to the natural killer effect. Attempts were made to generate "secondary" cytotoxic cells by cocultivating blood lymphocytes with autologous tumor biopsy cells. Cytotoxic cells were generated in 12 of 19 cases. In 11 cases tested, they reacted with the autologous tumor target and not with allogeneic tumors of similar or different histopathology.

The investigator studied the effect of interferon treatment of effector lymphocytes. Interferon treatment induced cytotoxicity in about 50% of the experiments against allogeneic tumor biopsy cells (49% of 37 experiments with tumor patients and 52% of 50 experiments with healthy donor lymphocytes). Interferon did not affect autologous lymphocytotoxicity.

Significance to Cancer Research: The question whether the human tumor patient reacts against and/or can be sensitized against his autologous tumor is the central problem of human tumor immunology.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Program
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $65,000

CONTRACT RESEARCH SUMMARY

Title: Characterization of Factors Causing Inhibition of Macrophage Function of Inflammatory Responses

Principal Investigator: Dr. D. S. Nelson
Performing Organization: Kolling Institute
City and State: St. Leonards, Australia

Contract Number: NO1-CB-84251
Starting Date: 9/30/78 Expiration Date: 9-29-81

Goal: To conduct research designed to functionally characterize factors produced by and/or induced by tumors, which impair macrophage function and/or inflammatory responses in vivo and in vitro.

Approach: Attempts will be made to evaluate the nature, extent, mode of action, and duration of impairment of macrophage function and inflammatory responses produced directly or indirectly by tumor cell products. Tumors will be of mouse, rat, and human origin. The effects of such factors on monocytes, stimulated and activated macrophages, and on tumor-associated macrophages will be compared. In addition, studies will be undertaken to assess the significance of such factors with respect to tumor growth in animals.

Progress: The key findings have been: (a) that supernatants from cultures of mouse tumors have inhibited delayed-type hypersensitivity reactions to sheep erythrocytes in the footpads of immunized mice over 17 weeks of age; (b) that macrophages from young and old mice are equally susceptible to inhibition of migration and phagocytosis by tumor produced factors in vitro; (c) that the factor inhibiting macrophage function in vitro is different from the factors with anti-inflammatory effects in vivo.

In limited experiments tumor supernatants also inhibited macrophage toxicity, spontaneous autoantibody formation and the production of lymphocyte activating factor. Immunization with supernatant material conferred partial resistance on an apparently unrelated tumor.

Significance to Cancer Research: There is increasing evidence that macrophages may have an important effector function to play in cell-mediated immunity both to infectious agents and tumors. Thus, an understanding of factors impairing macrophage function are of interest in the consideration of immunotherapeutic approaches to cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds $61,595

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Disseminated Human Cancer

Principal Investigator: Dr. Evan Hersh
Performing Organization: M.D. Anderson Hospital
City and State: Houston, TX

Contract Number: NO1-CB-33888
Starting Date: 6/25/73 Expiration Date: 7/7/81

Goal: To develop immunological treatments for patients with human cancer.

Approach: Phase I and Phase II trials are underway to evaluate various immunotherapeutic agents.

Progress: 1)Immune evaluation of 15 patients on a Phase I study receiving weekly IV-MER demonstrated significant boosting of antibody-dependent cell-mediated cytotoxicity to chicken red blood cells, increase in NK cells, increase in the numbers of adherent monocytes in peripheral blood, and increase in serum lysozyme. Suppressor cells were not generated by MER administration and a lymphocyte blastogenic response developed 1-2 weeks after the first dose. 2) A trial comparing chemotherapy (DTIC + Actinomycin D) with and without intensive intravenous C. parvum in patients with Stage IVB malignant melanoma demonstrated no significant difference between the two groups in response rate, response duration and overall survival in 65 evaluable patients. 3) A study is underway comparing patients with Stage II-III breast cancer treated with FAC chemotherapy alone or with BCG. There have been 10% relapses in both groups with a median follow up of 18 months. It is too early and there have been too few relapses in a total of 200 evaluable patients to detect a difference between the groups.4)At present time there is no significant difference in response rate, remission duration or survival between AML patients over the age of 50 who receive ROAP chemotherapy alone or those who receive ROAP chemotherapy plus BCG in a total of 65 evaluable patients. 5) A Phase I study of intratumoral BCG CWS plus P_2 plus endotoxin (ET) is being conducted. The dosage of ET was escalated while the dosage of BCG CWS+P_2 was constant. Seven patients have been treated a total of nineteen times and the most common side effects observed were erythema in the injection site, fever 100° and chills. Progressive toxicity has not been a problem with repeated doses or in two patients with prior BCG exposure. The highest dose of endotoxin given thus far was 1 ug. Limiting toxicity has not been reached. 6) Ten patients have entered a randomized immunopharmacological study designed to evaluate whether thymosin fraction V can correct T-cell immunodeficiency in patients with disseminated malignancy.

Significance to Cancer Research: It is important to confirm in prospectively randomized trials earlier studies showing immunotherapy to be of benefit to cancer patients.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $112,866

CONTRACT RESEARCH SUMMARY

Title: Chemoimmunotherapy of Acute Myelocytic Leukemia

Principal Investigator: Dr. James F. Holland
Performing Organization: Mt. Sinai School of Medicine
City and State: New York, NY

Contract Number: NO1-CB-43879
Starting Date: 6/30/74 Expiration Date: 3-20-81

Goal: To evaluate the usefulness of neuraminidase-treated allogeneic AML cells (VCN-AML) in acute myelocytic leukemia (AML).

Approach: Following remission induction with cytosine, arabinoside, and daunorubicin, AML patients had earlier been randomly assigned to maintenance chemotherapy alone, chemotherapy in combination with VCN-AML or in combination with VCN-AML plus MER. The MER arm has been deleted from the protocol. Patients are studied with both in vivo and in vitro immunologic tests.

Progress: In the current study 39 patients have been randomized to receive chemoimmunotherapy (immunotherapy consists of neuraminidase-treated allogeneic myeloblasts) and 37 to chemotherapy. A recent analysis was conducted on 69 of these 76 patients.

In the chemotherapy-alone group, 13 out of 34 patients (38%) remained in complete remission (CR) compared to 20 out of 35 (58%) in the group of patients receiving immunotherapy. Six out of 20 immunotherapy patients in CR are in the plateau phase at 19-30 months, while 1 out of 13 control patients are in remission past 19 months. Comparison of the remission curves at this early stage yield $p=.19$, $p=.13$, $p=.09$ by the Breslows, Logrank and Cox regression analysis respectively.

The investigators failed to detect any correlation, in either Study 1 or 2, between the initial skin test response to recall antigens and subsequent response to immunotherapy or the remission duration in any treatment group.

In vitro studies are underway to evaluate the lymphocyte number, function and response of remission lymphocytes to autologous myeloblasts over time.

Significance to Cancer Research: Preliminary data analysis suggests that the combination of sustaining chemotherapy with neuraminidase-treated allogeneic AML cells may represent a significant advance in the treatment of AML.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $141,467

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Treatment of Minimal Residual Systemic Tumor

Principal Investigator: Dr. Gerald L. Bartlett
Performing Organization: Pennsylvania State University
City and State: Hershey, PA

Contract Number: NO1-CB-33891
Starting Date: 6/30/73 Expiration Date: 10/31/80

Goal: To develop animal tumor models which will be useful for evaluating immune stimulants as immunotherapeutic agents.

Approach: In four different tumor-host models, animals are given immunotherapy at various times after tumor implantation, either alone or in combination with other means of treatment. The effect of the immunotherapy and the role of immune stimulants in immunotherapy are evaluated in terms of improved survival of treated animals. Animals which survive are tested further for improved tumor rejection immunity.

Progress: 13762A Rat Mammary Adenocarcinoma. Rats cured by surgery after day 14 show strong tumor immunity. Concomitant immunity has been used to demonstrate the kinetics of tumor immunity in the treatment protocol employed and to detect immunity-stimulating treatments. Viable tumor cells (10^8) are immunogenic, but irradiated tumor cells are not. In contrast, irradiated tumor cells can evoke DH but mitomycin C-treated cells cannot. Tumor immunity can be adoptively transferred by T-cells.

Line 10 Guinea Pig Hepatoma. L10X, which is an ineffective vaccine in male guinea pigs, is immunogenic for L10 in female guinea pigs that have been sensitized with a male skin graft.

CaD2 Mammary Adenocarcinoma. Treatment with antithymocyte serum abolishes the therapeutic effect of i.t. Cp but not the effect of i.v. Cp. Drug induced tolerance to Cp has been induced. DH tolerance to Cp does not interfere with any of the tumor inhibitory properties of Cp in this model.

LSTRA Mouse Leukemia Chemoimmunotherapy (CY + vaccine) continues to be inconsistent, even in replicate experiments. Optimal adjuvants and optimal route of injection increase the efficacy of immunity by accelerating the induction of effective resistance to challenge tumor. LSTRA immunity is abolished by either prevaccine or postvaccine treatment with either ATS or CY, or by continuous treatment with trypan blue.

Significance to Cancer Research: These studies demonstrate that caution must be exercised in drawing generalizations on immunotherapy from a single experimental or clinical result.

Program Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $270,888

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Mouse Ovarian Using Specific Serotherapy in Combination with Intraperitoneal C. parvum

Principal Investigator: Dr. Robert C. Bast
Performing Organization: Sidney Farber Cancer Center
City and State: Boston, MA

Contract Number: NO1-CB-74194
Starting Date: 9/30/77 Expiration Date: 9-29-80

Goal: To evaluate the therapeutic efficacy of specific xenogeneic serotherapy in combination with intraperitoneal C. parvum in the treatment of syngeneic murine ovarian carcinoma transplants.

Approach: Efforts will be made to determine the optimal dose, route, order and schedule of administration for the treatment combination in mice. Attempts will be made to determine relevant in vitro correlates with disease status. Techniques will be perfected for cryopreservation of human ovarian tumor cells and for the preparation of specific xenoantiserum against human tumor cells for possible use in human clinical trials.

Progress: Using somatic cell hybridization techniques, a monodonal murine IgG1 immunoglobulin has been isolated which binds to 6 of 6 epithelial human ovarian carcinoma lines and to cryopreserved tumor cells from at least 7 of 11 patients, but fails to react with adult and fetal ovary or with an epithelial cell line established from nonmalignant adult ovary. This antibody, designated OC 125, reacts by indirect immunofluorescence with 1 of 7 cell lines from nonovarian neoplasms and does not bind to a B lymphocyte cell line from the donor against whose cells the antibody was initially developed. Several human ovarian carcinoma cell lines can be lysed with OC 125 in the presence of activated murine peritoneal cells from C. parvum treated donors.

In a phase I study, C. parvum has been administered intraperitoneally to 3 patients with small, but visible deposits of ovarian carcinoma that remained following surgery and combination chemotherapy. The first person to receive i.p. C. parvum treatment has attained complete remission for at least 5 months. This remission was evaluated by a second look laparotomy, multiple biopsies and peritoneal washings. C. parvum augments the ability of human peritoneal effectors to lyse human ovarian carcinoma targets in the presence of specific rabbit xenoantiserum. In the absence of antibody, C. parvum activated human peritoneal cells detach or lyse > 90% of human ovarian carcinoma targets within 48 hours. The same activated cell populations inhibit proliferation of autochthonous peripheral blood lymphocytes in the presence of alloantigens.

Significance to Cancer Research: Synergy between two different forms of immunotherapy may permit more effective treatment of human cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Role of Circulating Tumor Antigens

Principal Investigator: Dr. Joseph D. Feldman
Performing Organization: Scripps Clinic and Research Foundation
City and State: LaJolla, CA

Contract Number: NO1-CB-43874
Starting Date: 3/1/75 Expiration Date: 2/28/80

Goal: To evaluate the effects of immunologic agents and reagents such as antigens, antigen-antibody complexes, and effector cells on the treatment of a virus-induced rat tumor.

Approach: Attempts will be made to augment and exaggerate the host immunologic responses that inhibit or destroy tumor in vivo and to suppress those responses that enhance tumor growth. This entails preparation of (a) membrane antigens of tumor cells; (b) cytotoxic antibodies of a single immunoglobulin class and specificity; and (c) purified populations of cytotoxic effector cells.

Progress: After infusion of syngeneic splenic cells with cytotoxicity augmented in a 7-day MLTC, established subcutaneous rat Moloney sarcoma tumors (MST-1) were completely and permanently eliminated. The effector cell associated with tumor elimination was defined as a T cell subset, W3/25+, W3/13+ (Williams monoclonal T antigen reagents), $Ia^{\pm}$, Ig^{-}.

A method was developed to separate antigens and antibodies of immune complexes circulating in tumor-bearing rats. Microgram quantities of antigen could be isolated and identified. Sera from rats bearing syngeneic and allogeneic tumors were examined for immune complexes and composition of complexes. Complexes of IgG and IgM were identified but tumor antigens, viral antigens and histocompatibility antigens were not detected.

Significance to Cancer Research: It is essential to understand the complex interactions of the components of the immune system and to determine how best to manipulate these interactions to achieve a therapeutic benefit in cancer treatment.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Phase I Study of Effects of Immune Stimulants on Human Immune Response

Principal Investigator: Dr. Yashar Hirshaut
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-53970
Starting Date: 5/16/75 Expiration Date: 5-15-81

Goal: To evaluate selected immunotherapeutic agents for acute and chronic toxicity, maximum tolerated dose, and effects on immune status.

Approach: Clinical protocols have been designed that provide for the administration of new immunotherapeutic agents to patients in accordance with acute chronic, and escalating dose schedules. Intravenous BCG, intravenous endotoxin and intravenous Poly IClc are currently under study.

Progress: Intravenous BCG in escalating doses has been given to 20 patients. The mean maximum tolerated dose was 2×10^6 VU. Limiting toxicity was usually high fever and chills. Following BCG treatment, BCG was cultured from the blood of 6 patients five minutes to three hours after treatment and was isolated from the tissues of two patients. BCG treatment appeared to increase the ability of LPS-stimulated neutrophils to reduce NBT; increase serum Clq and percent of Tu cells in peripheral blood; and increase in lymphocyte activating factor (LAF) release by LPS-stimulated adherent mononuclear cells.

Thirteen patients have entered in the intravenous Poly ICLC study in 14 individual trials. The mean maximum tolerated dose was 9.1 mg/M^2. Dose limiting toxicities include chills, hypotension, headaches and emesis. Marked leukopenia and thrombocytopenia were often marked but returned to normal after treatment was stopped. Poly ICLC was shown to induce significant levels of leukocyte interferon (mean peak serum level in 11 trials was 445 U/ml). Increases in natural killer cell activity and percent of Ia positive cells were noted in some patients.

Patient entry has recently begun on the endotoxin study.

Significance to Cancer Research: Immunotherapy is a new modality of treatment for cancer whose appropriate use depends on understanding of how available agents affect specific aspects of immune function. Phase I studies underway should assist in determining optimal doses producting acceptable toxicity.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $115,000

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Immunotherapy with Tumor Preparation in Man

Principal Investigator: Dr. Herbert F. Oettgen
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-64062
Starting Date: 6/16/76 Expiration Date: 6/15/80

Goal: To determine the effectivenes of treating patients with advanced malignant melanoma with a combination of surgery, BCG, autologous or allogeneic tumor cell vaccine, and chemotherapy (DTIC). To monitor the patient's immune response to surface antigens of autologous and allogeneic melanoma cells, as measured by serological and cellular reactions, and to correlate the response with the course of the disease and the effects of therapy.

Approach: Patients with malignant melanoma Stage III will be treated with BCG and a vaccine of irradiated tumor cells. Patients with malignant melanoma Stage IV will receive BCG, irradiated tumor cells, and DTIC. The patient's immune response to surface antigens of their own tumor cells will be tested in vitro by serological tests (mixed hemadsorption, immune adherence, complement-dependent cytotoxicity, antibody-directed cell-mediated cytotoxicity), and tests for lymphocyte cytotoxicity on autologous or allogeneic target cells.

Progress: Twenty-two patients have entered the autologous vaccine protocol and 23 patients have entered the allogeneic melanoma cell vaccine protocol. Extensive adsorption analysis in the serological study of these patients is underway. Particular emphasis has been placed on further analysis of the Class I and Class II antigenic systems. One new Class II antigenic system has been defined. In addition, investigation of the role of fetal calf serum in Class III reactions has been extended. Three additional cases of FCS-related Class III reactivity have been identified in the vaccinated group of patients.

Significance to Cancer Research: Malignant melanoma offers unique opportunities for study in that the serology is further advanced than in other types of cancer. Thus a trial of autologous vaccine will provide information concerning therapy as well as basic human cancer immunology.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunization with BCG and Allogeneic Renal Cancer Cells in Patients with Renal Cell Cancer

Principal Investigator: Dr. Herbert F. Oettgen
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-74145
Starting Date: 9/30/77 Expiration Date: 9-29-81

Goal: To determine whether the cells of renal cancers have cross-reacting tumor-associated antigens.

Approach: Assays for humoral immunity to autologous tumor cells will be performed before and after immunization with BCG and allogeneic renal cancer cells. Attempts will be made to analyze the specificity of the serological reactions and to investigate the patterns of antigen expression in terms of culture time, growth characteristics, and cell cycle kinetics. Periodic assessment of tumor immunity will be performed on sera from patients with Stage I renal cancer receiving conventional therapy and on sera from patients with Stage II, III, and IV renal cancer who are immunized with BCG and allogeneic renal cancer cells.

Progress: Twenty-one patients have been treated with allogeneic renal cancer cell vaccine and BCG. The choice of cell lines for the preparation of vaccines was based on a satisfactory growth rate and on the expression of shared renal cancer cell surface antigens. Of 12 patients who have been sufficiently tested, 10 showed a rise in the titer of antibodies against autologous renal cancer cell surface antigens. The specificity of these antibodies is under study. A higher frequency of antibody to shared (class II) antigens was observed in comparison to similar studies of melanoma, astrocytoma and leukemia.

To provide target cells for these studies, renal cancer tissue from 150 patients and normal kidney tissue from 96 patients have been placed in culture. Short term cultures (up to 4 passages) were established from 56 renal cancers (37%) and 88 normal kidneys (91%). Continuous cell lines were established from 27 renal cancers (18%).

Significance to Cancer Research: The interest in clinical immunotherapy has emphasized the need for specific and reproducible methods for monitoring the cancer patient's immunological reactivity to cancer cells. In addition, serology appears perhaps the most direct approach to determining the form and source of tumor antigens to be used in clinical trials of active immunotherapy for cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $133,086

CONTRACT RESEARCH SUMMARY

Title: Evaluation of C. parvum in Disseminated Carcinoma of the Breast

Principal Investigator: Dr. Carl M. Pinsky
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-53873
Starting Date: 6/30/75 Expiration Date: 6/29/80

Goal: To determine whether the addition of Corynebacterium parvum to combination chemotherapy in patients with advanced breast cancer increases response rate, increases response duration or survival time, protects against the deleterious effects of chemotherapy, or augments immunologic function.

Approach: This is a prospective, randomized trial using cyclophosphamide, adriamycin, methotrexate, and 5-fluorouracil every 28 days, with or without C. parvum given weekly, subcutaneously.

Progress: One hundred and twenty patients have entered the randomized study. Of 107 evaluable patients, a partial remission was seen in 28 of 53 patients (53%) who received CAMF and in 20 of 54 patients (37%) who received CAMF + C. parvum (P = N.S.). One patient in each treatment group had a complete remission.

Comparing the patients who received CAMF + C. parvum, respectively, estimated median response duration was 15 vs 12 months, estimated overall survival of responders was 20 vs 24 months. None of these differences was statistically significant. Toxicity and immunologic reactivity was also similar in the two groups. Though earlier analyses had yielded advantages for the CAMF + C. parvum group in response duration or survival, no differences are apparent at this time.

Significance to Cancer Research: Since complete response rate is minimal and ultimate survival continues to be short in patients with advanced breast cancer, all leads which suggest lengthened survival must be followed.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: BCG Immunotherapy in Patients with Recurrent Superficial Bladder Cancer

Principal Investigator: Dr. Carl M. Pinsky
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-74146
Starting Date: 9/16/77 Expiration Date: 9-15-81

Goal: To evaluate the therapeutic efficacy of intravesical and percutaneous BCG in patients with recurrent superficial bladder cancer.

Approach: A controlled, randomized, prospective study will be undertaken in which the administration of BCG shall be identical to the methods published in Journal of Urology 116:180-183, 1976. The study will assess the effect of BCG therapy on the rate of tumor recurrence.

Progress: A total of 51 patients have been randomly assigned to treatment in this study: 25 on the BCG arm and 26 on the control arm. The mean age is 59, and the ages range from 24 to 77 years. The patient groups were comparable with respect to sex, pre-randomization urine cytology and pre-randomization treatment. There has been no severe toxicity seen in the BCG-treated patients to date, but dysuria, hematuria, urinary frequency and urgency have been commmon. Preliminary results suggest that the patients treated with fulgur - ation plus BCG have a longer time to recurrence, fewer tumors, and more frequent conversion of cytology from positive to negative than the patients treated with fulguration alone.

Significance to Cancer Research: A pilot study has suggested that intravesi- cal and percutaneous BCG decreases the number of recurrences in patients with superficial bladder carcinoma. It is important to establish that this is the case with a controlled, prospective, randomized trial.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $52,978

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Levamisole as a Therapeutic Adjunct in Squamous Cell Carcinoma of the Head and Neck

Principal Investigator: Dr. Elliot W. Strong
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-53875
Starting Date: 6/30/75 Expiration Date: 6/29/83

Goal: To evaluate the usefulness of levamisole in the treatment of patients with squamous cell cancer of the head and neck.

Approach: Patients stratified according to type of disease, primary or recurrent, stage and site, are randomized after surgery on a double-blind basis to receive levamisole or a placebo. Treatment is given three times on alternate weeks for two years. The effects of levamisole on clinical course as well as on immune status are evaluated.

Progress: One hundred nineteen patients have been entered on study of which 104 are evaluable. All evaluable patients had squamous cell carcinoma of the oral cavity, pharnyx, or larynx and were free of detectable cancer after surgery for recurrent or primary disease. They were randomized to receive Levamisole or placebo (52 evaluable patients in each arm). There is no difference in time to recurrence between the patients who received Levamisole and those who received placebo. In the group of 19 patients with primary operable disease, stage 2, there is a statistically significant prolongation of time to recurrence for the group who received Levamisole. This result must be viewed with caution due to the small number of patients and relatively short follow-up time. When stratifying for stage or treatment arm, no significant difference was found in remission duration for patients initially DNCB positive compared to those patients initially DNCB negative.

Significance to Cancer Research: Immunopotentiation is a promising clinical approach to cancer therapy especially when used as an adjunct in patients with known immunosuppression. Levamisole, an immune modulator, has been reported to be useful in the treatment of resectable lung cancer and in patients treated for locally advanced breast cancer with radiation (Rojas et al.). This study will attempt to determine whether levamisole has therapeutic efficacy in patients with head and neck cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $85,008

CONTRACT RESEARCH SUMMARY

Title: Specific and Non specific Immunotherapy as an Adjuvant to Chemotherapy

Principal Investigator: Dr. Frederick Eilber
Performing Organization: University of California
City and State: Los Angeles, CA

Contract Number: NO1-CB-04344
Starting Date: 6/16/75 Expiration Date: 6/15/83

Goal: To determine if post-surgical adjuvant chemoimmunotherapy is superior to adjuvant chemotherapy in preventing disease recurrence in patients with highly malignant skeletal or soft-tissue sarcomas.

Approach: Patients with osteosarcoma or grade III soft-tissue sarcoma (rhabdomyosarcoma, synovial cell or anaplastic sarcoma) following complete surgical excision of their tumor are randomized between chemotherapy--adriamycin, high-dose methotrexate and citrovorium rescue--and identical chemotherapy plus immunotherapy consisting of BCG and allogeneic tissue culture sarcoma tumor cell vaccine. Chemotherapy is given for one year; the immunotherapy is given for the same year plus an additional year.

Progress: A total of 118 patients have entered this study, 64 who are receiving adjuvant chemotherapy and 54 who are receiving adjuvant chemo-immunotherapy therapy. Of these 118, 54 have stage I skeletal sarcoma.

Although the number of patients in each group is insufficient for statistical comparison, there does appear to be a benefit of immunotherapy combined with chemotherapy for stage I skeletal sarcomas. Seventeen of 25 (68%) of patients treated with chemo-immunotherapy remain continually free of disease whereas only 13/28 (46%) of patients treated with chemotherapy alone remain disease-free. The follow-up interval in the two groups is comparable, but the number of patients is too small for accurate analysis.

There does not appear to be a significant difference in the total number of courses of chemotherapy or the total amount of drug administered between the group receiving chemotherapy and the group receiving chemoimmunotherapy.

Significance to Cancer Research: The significance to this project has been to reduce disease recurrence and to prolong survival in a group of diseases that was previously rapidly fatal. Since the chemotherapy is discontinued after one year, it will be of major importance to determine if those patients who continue to receive immunotherapy will have a more lasting disease-free status than those patients receiving the chemotherapy alone.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $90,360

CONTRACT RESEARCH SUMMARY

Title: Human Melanoma: Evaluation of BCG Immunotherapy of Patients Without Detectable Disease After Removal of Tumor-containing Lymph Nodes

Principal Investigator: Dr. Donald L. Morton
Performing Organization: University of California
City and State: Los Angeles, CA

Contract Number: NO1-CB-64076
Starting Date: 8/8/74 Expiration Date: 10-7-80

Goal: To determine whether immunotherapy with BCG alone or BCG combined with tumor cell vaccine will decrease recurrence rate or prolong survival in melanoma patients with metastases to regional lymph nodes.

Approach: Patients are randomized following surgical lymphadenectomy into 3 groups receiving different postoperative therapy: one group receives no additional therapy, one receives BCG, and one group receives BCG and allogeneic tumor cell vaccine.

Progress: Recurrences were least frequent in the vaccine treated group (23 out of 47 - 49% compared to 27 out of 46-59% in the control group and 26 out of 45-57% in the BCG treated group). The differences between these recurrence frequencies is not, at present, statistically significant.

Patients receiving BCG immunotherapy who recurred are still surviving longer than those in the control group, or those patients receiving BCG plus tumor cell vaccine.

A blind analysis of stage II melanoma patients' sera for antibody against M14 did not confirm a previous observation that a modest rise in antibody levels following surgery correlated with a favorable prognosis. However, certain correlations could be made with the disease free interval and survival when absolute levels of antibody were measured in the preoperative period. The mean titer of anti-M14 IgM antibodies was higher in the group of patients remaining tumor free for two years relative to that in patients in whom disease recurred within two years.

A rise of complement fixation antibody titer greater than threefold in patient's receiving BCG with or without tumor cell vaccine was characteristic of patients whose disease did not recur within 30 months of entering the trial. Patients whose disease recurred with 6 to 12 months did not show such a rise in CF antibody titer.

Significance to Cancer Research: It is important to confirm in prospectively randomized trials earlier studies showing immunotherapy to be of benefit to cancer patients.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $146,641

CONTRACT RESEARCH SUMMARY

Title: Production of Monospecific Antibodies Against Human Malignant Lymphoma and Leukemia Tumor-associated Antigens

Principal Investigator: Dr. Ivor Royston
Performing Organization: University of California
City and State: San Diego, CA

Contract Number: NO1-CB-84250
Starting Date: 9/30/78 Expiration Date: 11/29/80

Goal: To produce xenogeneic monospecific antibodies to human lymphoma and leukemia associated antigens.

Approach: Attempts will be made to fuse antibody-producing cells of mouse origin with mouse myeloma cells in order to produce hybrid cell lines capable of continuous production of monospecific antibodies against tumor-associated antigens in vitro. Two different hybridization techniques will be compared. The specificity of the presumed monoclonal antibodies and the pattern of antigenic tumor-specific determinants on malignant lymphoid cells will be evaluated.

Progress: The investigator has generated a number of new hybridoma antibodies against human lymphoid leukemia and lymphoma cells some of which appeared at first to discriminate between the malignant and normal cells. However, these apparently unique antigens can be found on some normal lymphoid cells under the appropriate conditions.

The spleen fragment culture technique of Klinman offered no advantage over the hybridoma technique in terms of producing discriminating monoclonal tumor-associated antibodies. The investigators were able to obtain antibody-producing hybridomas by fusing small numbers of cells from $1mm^3$ polyclonal antibody-producing spleen fragments from directly immunized mice; but were unable to obtain hybridomas from monoclonal antibody-producing spleen fragments from irradiated recipients which had received an adoptive transfer of immune spleen cells.

Significance to Cancer Research: Large-scale production of monospecific anti-tumor cell antibodies produced in vitro may have significant application to immunotherapy and immunodiagnosis.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds:

CONTRACT RESEARCH SUMMARY

Title: Studies of Immune Stimulants in Patients Receiving Radiation Therapy

Principal Investigator: Dr. William M. Wara
Performing Organization: University of California
City and State: San Francisco, CA

Contract Number: NO1-CB-64004
Starting Date: 6/1/76 Expiration Date: 11-30-80

Goal: To document the immunosuppression which occurs following radiation therapy and to determine if a therapeutic advantage can be achieved by the addition of immunotherapy (thymosin) to the conventional treatment given for squamous cell carcinoma of the head and neck region and the esophagus.

Approach: Both groups of head and neck and esophageal cancer patients will receive radiation therapy and/or surgery as treatment. Patients will then be randomized to receive thymosin or placebo in addition to standard treatment. All patients will be monitored with immunologic tests to measure antibody-mediated and cell-mediated immunity at regular intervals. The data will be analyzed for local disease recurrence, metastatic disease, and survival difference in the patient groups.

Progress: Over 250 controls have been evaluated and their data accumulated to ascertain quality control of the laboratory results. One hundred thirty seven patients have been studied and their preliminary results indicate the expected immunosuppression after irradiation.

Following radiation therapy the administration of thymosin did not appear to affect the total number of lymphocytes, total T lymphocytes, and B-lymphocytes. Head and neck cancer patients were normal pre-irradiation with respect to lymphocyte stimulation with PHA. Immediately, post irradiation the thymosin-treated patients showed less suppression than the patients not treated with thymosin, but the values for both groups were suppressed from the pre-irradiation levels. With respect to lymphocyte stimulation by allogeneic cells (MLC), a significant difference is reported between the thymosin treated patients and those not receiving thymosin; the thymosin group retained their MLC capacity after immuno-suppressive irradiation. At three months post irradiation the differences in the PHA and MLC responses between the thymosin-treated and untreated patients became less apparent.

Significance to Cancer Research: This study will determine the significance of immunosuppression caused by irradiation in head and neck and esophageal cancer patients and whether the effect of immunotherapy (thymosin) in reversing this immunosuppression has any impact on disease-free interval, survival, and local control of cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $45,178

CONTRACT RESEARCH SUMMARY

Title: Intralesional Immunotherapy Prior to Surgery in the Treatment of Canine Breast Carcinoma

Principal Investigator: Dr. Steven Harris
Performing Organization: University of Texas
City and State: San Antonio, TX

Contract Number: NO1-CB-84248
Starting Date: 9/26/78 Expiration Date: 9-29-81

Goal: To evaluate in a controlled clinical study the effect of intralesional injection of BCG crude cell walls on canine breast carcinoma.

Approach: Dogs clinically free of detectable metastatic disease shall be randomly assigned to intralesional placebo prior to surgery or intralesional immunotherapy prior to surgery. The BCG crude cell walls will be provided by the National Cancer Institute. Tumor regression, tumor recurrence, disease-free interval, and survival data from the two groups shall be compared. Selected assays of humoral and cellular immunity shall be performed.

Progress: The randomized Phase III study was begun using an equal ratio between placebo and BCG-CW groups. So far ten dogs have entered each of these two groups. An additional 11 dogs whose owners refused the randomized trial are also being followed to observe the effects of surgery alone. Including the earlier Phase I trial, a total of 70 dogs have been studied. Developmental work continued on the CMI assay. The humoral assay showed that all dogs treated with BCG-CW developed and maintained a measurable response to BCG-CW. Yet PPD skin tests in the few animals on protocol for one year remained negative. Histological examination of biopsy and surgical specimens showed a marked inflammatory response and destruction of adenocarcinoma elements in the lesions injected with BCG-CW as compared to the placebo group. It is too early to assess survival data.

Significance to Cancer Research: In considering the optimal setting for immunotherapy in the treatment of human cancer, it is important to assess the efficacy of intralesional immunotherapy prior to surgery in spontaneous tumors of outbred animals with limited disease.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $89,009

CONTRACT RESEARCH SUMMARY

Title: BCG Immunotherapy of Recurrent Superficial Bladder Cancer

Principal Investigator: Dr. Donald L. Lamm
Performing Organization: University of Texas
City and State: San Antonio, TX

Contract Number: NO1-CB-74192
Starting Date: 9/3/77 Expiration Date: 3-31-80

Goal: To evaluate the therapeutic efficacy of intravesical and percutaneous BCG in patients with recurrent superficial bladder cancer.

Approach: A controlled randomized prospective study will be undertaken in which the administration of BCG shall be identical to the methods published in Journal of Urology 116:180-183, 1976. The study will assess the effect of BCG therapy on the rate of tumor recurrence.

Progress: Fifty-one patients have been enrolled in this study. Twenty-four patients were randomly assigned to the BCG treatment group and 27 to control. The observed side effects of BCG therapy were mild dysuria, urinary frequency, mild gross hematuria, fever (less than 101°F) and nausea and vomiting. No patient has required postponement of BCG immunotherapy due to side effects.

Twenty-three BCG-treated patients and 24 controls have been followed for 3 to 24 months. Eleven of 24 control patients (46%) have developed recurrent tumors compared with 5 of 23 BCG-treated patients (22%). Nineteen episodes of tumor recurrence with a total of 67 individual tumors occurred in the control group compared with 8 episodes of recurrence and a total of 17 tumors in the BCG group. Mean time to recurrence for the control group is 12 months compared with 19.4 months for the BCG treatment group ($p=0.023$ Wilcoxon).

Significance to Cancer Research: A pilot study has suggested that intravesical and percutaneous BCG decreases the number of recurrences in patients with superficial bladder carcinoma. It is important to establish that this is the case with a controlled, prospective randomized trial.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTROL RESEARCH SUMMARY

Title: Adoptive Cellular Immunotherapy of Murine Tumors

Principal Investigator: Dr. Alexander Fefer
Performing Organization: University of Washington
City and State: Seattle, WA

Contract Number: NO1-CB-84247
Starting Date: 9/30/78 Expiration Date: 9/29/80

Goal: To determine the conditions and operative mechanisms for achieving optimal therapeutic effect of adoptive cellular immunotherapy used alone or in combination with chemotherapy in the treatment of several mouse tumors.

Approach: Attempts will be made to utilize in adoptive cellular immunotherapy experiments cells immunized both in vitro and in vivo. Studies will be undertaken to characterize the effector cell(s) responsible for tumor therapy as well as cells which suppress the therapeutic effect. The role of the antigens for the major histocompatibility complex in sensitization to TAA and in the generation of cells therapeutically effective against established syngeneic tumors will be explored.

Progress: Adoptive immunotherapy and/or adoptive chemoimmunotherapy models have been established for EL-4 (G-) and FBL-3 tumors in C57Bl/6 mice, LSTRA tumor in Balb/c mice, and FBL-3 and LSTRA tumors in (Balb/c C57Bl/6) Fl mice. Studies have been conducted to evaluate the specificity and therapeutic efficacy of the adoptively transferred cells which have been sensitized primarily or secondarily in vitro.

Cell separation studies are underway to identify the effector and suppressor cell subpopulations in cultured cells used for adoptive immunotherapy.

The role of antigens of the major histocompatibility complex in sensitization to tumor antigens, in vitro lysis of tumor cells, and in vivo eradication of tumor is being assessed. Preliminary experiments were conducted with LSTRA ($H\text{-}2^d$) and FBL-3 ($H\text{-}2^b$) tumors in (Balb/c x C57Bl/6) Fl mice. Cells secondarily sensitized in vitro were tested in vivo in adoptive chemoimmunotherapy of established tumor and demonstrated H-2 restriction. However, if the immune cells used in therapy were sensitized in vivo with these parental tumors, immune cells demonstrated a preference for the sensitizing parental haplotype but not the apparent restriction observed following secondary in vitro sensitization.

Significance to Cancer Research: The cellular component of the immune system has been demonstrated to be of importance to the rejection of transplanted syngeneic tumor in mice. This research addresses many of the theoretical and technical problems attending the use of adoptively transferred cells as a treatment for malignancies.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds:

CONTRACT RESEARCH SUMMARY

Title: Intratumoral BCG Immunotherapy Prior to Surgery for Carcinoma of the Lung

Principal Investigator:	Dr. Richard Matthay
Performing Organization:	Yale University
City and State:	New Haven, CT
Contract Number:	NO1-CB-74191
Starting Date:	9/30/77
Expiration Date:	9-29-81

Goal: To evaluate the therapeutic efficacy of intratumoral BCG prior to surgery for carcinoma of the lung.

Approach: Patients with potentially resectable carcinoma of the lung will be randomly assigned to treatment with intratumoral BCG (administered by transthoracic needle or fiberoptic bronchoscope) two weeks prior to surgery or surgery alone. Survival and disease-free interval will be analyzed in the two treatment groups.

Progress: A total of 57 patients have entered this study with 32 in the BCG group and 25 in the control group. Thirty-nine of the 57 patients are alive and disease free without recurrence. There have been 18 recurrences and/or deaths: 9 among Stage III BCG-injected patients, 5 in Stage III control patients and 2 each in Stage I BCG and Stage I control patients, respectively. All BCG and control Stage II patients are disease free.

In the BCG-treated patients the maximum post injection temperature ranged from 100.8° to 104°F (average 102.6°F). In 27 of the 32 BCG injected patients a transient, peritumoral radiographic infiltrate was noted and histologic evidence of tuberculous pneumonitis was present in 23 cases. This reaction did not impair resectional surgery or wound healing in any case. In eight cases, acid fast bacilli were seen and/or grown from surgical material. All BCG-treated patients, except one, were injected bronchoscopically. One individual was injected transthoracically.

Significance to Cancer Research: Relatively little is known about the value of immunotherapy prior to surgery in early clinically nondisseminated human malignancies. It is important to assess this therapeutic approach in a controlled, randomized prospective study.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $81,750

ANNUAL REPORT OF THE
BREAST CANCER PROGRAM COORDINATING BRANCH, DCBD
NATIONAL CANCER INSTITUTE

October 1, 1980 - September 30, 1980

Program Description: The general aims of this Branch and the Breast Cancer Task Force Committee (BCTF) of extramural advisors are to promote multidisciplinary research projects in experimental biology and clinical medicine that will lead to improved diagnosis, prognosis, treatment and prevention of breast cancer. The Branch activities are divided into five Sections: Diagnosis, Epidemiology, Experimental Biology, Therapeutics and Information. The members of the Task Force Committee are selected for their training and experience related to the activities of the first four Sections named. The research projects initiated, supported, and monitored are extramural, they are funded through grant and contract mechanisms. Members of the Committee provide advice on new programs to be initiated based on their knowledge of: 1) ongoing research sponsored by the NCI and other organizations; 2) information obtained at workshops and conferences sponsored by the Committee and the Branch; 3) their own research and that of their colleagues in their institutions. The Staff of the Branch prepare requests for grant applications (RFA), Program Announcements, and requests for contract proposals (RFP) based on the advice of the Committee members and these require approval by an intramural Steering Committee of the Division of Cancer Biology and Diagnosis.

Activities: During the past year the staff and our advisors have continued to emphasize the grants program for conducting breast cancer research. Two RFAs and two Program Announcements have been published, the titles are listed in Table I. Five grant applications were received in response to the two Announcements however none were funded in FY 1980 because of the Study Section review cycle in which they were received. The receipt deadlines for the two RFAs were such that they will not be reviewed until FY 1981. The Program Announcement on genetic susceptibility resulted from a Workshop sponsored by the Diagnosis Section and Committee members in FY 1979, three of the five applications reviewed were for this Announcement. Three Workshops were sponsored in FY 1980 (Table I) as an additional means of stimulating new research projects while reviewing the state-of-the-art on the respective subjects. Also an overview meeting of the breast cancer program was held July 28-29, 1980. Oral presentations were made by investigators sponsored by grant and contract funds. The third day a Task Force Committee meeting was devoted to an evaluation of the program presented and suggestions for new RFAs, Program Announcements and RFPs to be considered by staff in FY 1981.

The BCTF Committee membership was reduced from 56 members to 38 members in FY 1980 by order of the Government to diminish the use of consultants. There will be a further reduction in FY 1981. A new extramural chairman was selected and this, too, became necessary because of a Government regulation. The meetings of the Committee were curtailed because of a shortage of travel funds. Fortunately the Chairman and the members were willing to work with Staff by phone, correspondence and on occassions when they were in Bethesda.

TABLE I

RFAs, Program Announcements and Workshops
Sponsored by the BCTF Committee and the BCPC Branch in
FY - 1980

Program Announcements

1. Experimental Research Related to Mammographic Screening for Human Breast Cancer. (Experimental Biology and Diagnosis Sections)

2. Research Related to Genetic Susceptibility to Human Breast Cancer. (Epidemiology Section)

RFAs

1. Studies of Immunocompetent Cells Infiltrating Human Breast Cancer. (Diagnosis Section)

2. Correlation Between Microscopic Characteristic of Primary Breast Tumors and Subsequent Patient Survival. (Epidemiology Section)

Workshops*

1. Polypeptide Hormone Receptors in Normal and Malignant Breast Tissue. November 13-14, 1980 (Diagnosis Section)

2. Minimal Breast Cancer. March 11-12, 1980 (Therapeutics and Diagnosis Sections)

3. Culturing Human Mammary Epithelial Cells. April 22-23, 1980 (Experimental Biology Section)

*The meetings were open to all interested persons.

The number of investigator-initiated projects increased during FY 1980 to 46, totalling approximately $5 million. Of the total number two were Program Project grants, one in Epidemiology the other in Esperimental Biology; one Conference grant; and one was a New Investigator Award. The remainder of the projects were the regular RO-1 grants competitive and non-competitive. At the conclusion of this report a list of the grants and contracts by Section is presented, these are categorized by scientific focus or emphasis. Grants are listed by number, title, investigator and institution; contracts are listed only by number, investigator and institution, a research summary of each is included in this report.

Contract funding decreased in FY 1980, $3.1 from $6.3 million. Although 80 contracts summaries are provided only 41 were funded in the fiscal year, the remainder were terminated during the year or extended without funding in order to obtain final and meaningful data. A contract project entitled "Evaluation of the Impact of the Estrogen Receptor Assay on the Treatment of Human Breast Cancer" was advertised in FY 1980 by the Therapeutics Section. The goals for this research service project are to determine the extent that estrogen assays are being performed on breast cancer tissues and the extent that the assay results influence clinical management of the patients with the disease. Funds for the project resulted from competing for evaluation set-aside funds from the Office of the Director, NIH. Thirteen responses to the RFP were received, however, only one could be funded with the dollars available. The high caliber of the proposals, the investigators and their institutions were unanticipated.

Diagnosis Program: There were five targeted approaches (listing appends this report) that included 17 contracts and six grants. Thirteen contracts were research oriented and four were for obtaining resource material (serum samples). The six grants were investigator-initiated research.

Three of the research projects (CB-43869, 84237 and CA25836) were concerned with the evaluation of a non-invasive biophysical approach to breast cancer detection and diagnosis: thermography, ultrasound and radionuclide localization respectively. The first two listed projects were terminated. The final report was received on the long term (CB-43896) contract in which the use of computerized, automated absolute temperature thermography with pattern recognition technique were investigated as a valuable prescreening device, and, the investigator (J. Haberman) presented the results at the Overview meeting. Although voluminous data were obtained the usefulness of this technique for prescreening was judged doubtful. The clinical evaluation project on two ultrasonic machines as a possible alternative or adjunct to x-ray mammography (CB-84273) was terminated on the basis of inadequate analysis of the data and the kind and intensity of statistical support that was needed to arrive at defensible conclusions on the results of the study. The one grant in this targeted approach is concerned with the synthesis and evaluation of receptor-based selective breast tumor localizing agents whose distribution can then be monitored in a non-invasive way by γ-imaging techniques. Dr. Katzenellenbogen reported excellent progress in the preparation of compounds at the overview meeting.

There are three contracts and two grants involved in immunodiagnostic studies. Under contract CB-84224 Dr. Medof continues to assay levels of immune complexes in sera of breast cancer patients to ascertain how they correlate with diagnosis and prognosis. Dr. Chu (CA25653) is conducting a similar study, his data appear promising, sera of breast cancer patients have shown significantly higher Clq binding activity than sera from patients with gross fibrocystic disease. Also Chu and colleagues have demonstrated that the isoelectric focusing technique represents an effective approach to the dissociation and characterization of these antigen-antibody moities. Contract CB-74087 has terminated, the objective was to ascertain whether the leukocyte inhibition (LMI) assay was useful for the diagnosis and/or prognosis of breast cancer, utilizing human peripheral blood leukocytes for the detection of cell-mediated immunity against MMTV antigens. Data showed that the test appears to have little value for early diagnosis, but may be of some value for monitoring of disease progression. The third contractor (CB-84228) has continued investigating the relevance of regional lymph node immune response to prognosis of the disease. Evidence has been found for numerous alterations within the immune system of breast cancer patients. There are marked differences in some breast cancer patients' effector cell populations as well as in proliferative response and lymphokine production of lymph node cells compared to controls, indicating tumor related immune stimulation. In the continuing grant CA19083, to confirm and apply a previous finding that Thomas-Friedenreich (T) antigen is adenocarcinoma associated, and, that human breast patients show cell-mediated as well as human immune responses in vivo and in vitro to T antigen, whereas healthy persons do not, the results to date are striking but must be confirmed by other laboratories.

The search for biochemical markers in breast cancer patients has continued. Four contracts and one grant have been involved in the search. Three contracts (CB-53853, 74206, 74086) have terminated except for patient follow-up. Dr. Schwartz is principal investigator on two of the contracts (CB-53853, 74206), in the earlier projects he completed an intense biochemical examination of breast cyst fluid for markers that might be useful in understanding the function of cysts and their role in development of breast malignancies. Patients are being followed for correlation with breast cancer development. In the second project, longitudinal marker studies are performed on the sera and tissue of breast cancer patients prior to mastectomy and throughout the clinical course of the disease to determine how effective these markers would be for early diagnosis and recurrence, and, to gain an understanding of the relationship between the concentration of the marker substance in the individual tumor and in the serum levels of the host. Preliminary evaluations have indicated a number of differences between normal and cancerous breast tissue. Markers that are elevated at various times during the clinical course of the disease have been observed. The objectives of the third contract (CB-74086) have been similar to the one just described. Dr. Sussman has found in postmenopausal patients the alpha hCG levels in metastatic patients significantly higher than levels in the primary patients and controls. He has suggested that combining the alpha, beta hCB and PAP levels might be a way to discriminate between postmenopausal patients who will have metastases and those who will not. The fourth contract (CB-84222) and the one grant (CA25574) are closely related in objectives. Under the contract various isoenzymes or isoproteins are being detected, quantitated, and characterized in

normal, benign, and neoplastic breast tissue in an attempt to identify markers for early detection and/or identification of patients with occult metastases. The grantee, Dr. Yang, is studying the effect of mammotropic hormones on differential isoenzyme expression, whether these isoenzyme differences are detectable in breast fluids, and whether this is useful for the diagnosis of breast cancer. He is utilizing tissue culture systems of normal and malignant mammary epithelium. He has reported that over 60 percent of breast tumors showed an increase in LDH^5 as compared to normal breast fluids. Now he is working to establish these LDH^1/LDH^5 differences quantitatively. Two other research contracts (CB-84316, 84223) are attempting to characterize potentially premalignant epithelial hyperplasias of human breasts biochemically, morphologically and immunologically for identification of markers of malignant potential. Dr. McCarty, Jr. has observed a localization of immunoglobulin G in a pericellular pattern in dysplastic epithelial hyperplastic lesions, while apical IgA localization has characterized the non-dysplastic benign lesions. Dr. H. Jensen is working to establish whether angiogenesis is a reliable criterion for precancer and to characterize by biochemical and immunological markers the type of lesions that possess angiogenic capacity from those that do not. She has found that adenomas, developing lobules, and lactating lobules are commonly angiogenic while fibroadenomas, apocrine cysts, normal ducts and most normal lobules are non-angiogenic. Angiogenesis appears to pinpoint atypical lobules in women with, are at high risk for breast cancer.

Two contractors and two grantees were and are involved in a search for morphological discriminants as indicators of high risk for malignant disease. Both contracts have terminated (CB-74098,74097). Dr. Page was assessing the risk associated with human in situ carcinoma and breast hyperplasias. Data from almost 2000 completed follow-ups and 6000 reclassified histological slides have been analyzed, the final results will be reported in the coming months. Dr. Rosen sought to identify clinical and pathological prognostic factors for disseminated disease in patients with positive nodes and in those with negative nodes who had early invasive disease. He located 134 cases with a lesion of 1 cm. or less and 248 cases in the 1.0 to 2.0 cm. category. Analysis of risk factors has revealed to date that patients with intralymphatic tumor emboli of the breast had a significantly higher likelihood of recurrence than those who did not exhibit the lesion. The project of the grantee supported by the New Investigator Fund (CA26406) has the objectives of delineating specific cellular markers for distinguishing the different types of epithelial cells in normal, dysplastic and malignant mammary tissues. She will attempt to identify and characterize cell surface factors which may be involved in mediating the interactions of these cells with each other and with the extracellular substrate to which they attach. The second grantee (CA28128) seeks to more fully characterize blood group antigenic changes that occur with neoplastic transformation. He is utilizing immunohistological techniques on formalin-fixed slides.

The last category of contracts are resource oriented, three medical centers are processing serum samples from: 1) asymptomatic women; 2) women with benign breast disease; 3) breast cancer patients. The samples are shipped to a storage facility at Mayo Foundation where they are coded for future use of investigators conducting biological marker studies. In FY 1981 these contracts will be in the Therapeutics Section.

Epidemiology Program: Twenty-one contracts and three grants are categorized in eight targeted approaches to epidemiological problems of breast cancer. Several of these are listed under more than one category. Six (CB-84274, 53884, 63998, 84319, 84238, 84317) of the contracts were terminated in FY 1980 and several were extended without funds. One of the grants (CA13556) is a Program Project that was transferred to the Program because of its strong breast cancer emphasis. The overall focus of the project is not simply statistical, descriptive epidemiology but an exploration of etiology involving a broad multidisciplinary approach.

In the area of genetic susceptibility one terminated contract, CB-84274, was the source of a major breakthrough that has received nationwide attention in recent months. This was the discovery of a linkage, in some but not in all families with a high incidence of breast cancer, between breast cancer and one variant of the gene for glutamine pyruvate transaminase (GPT-1), thus localizing one gene for susceptibility on chromosome 10. The finding opened up an entire new realm of follow-up research including of course the continued attempt to localize other genes for susceptibility through similar linkage analysis. The investigator responsible for the work was awarded a grant in FY 1980 to continue her work (CA27632).

Emphasis on the endocrinology of breast cancer was continued through a variety of studies: A. Looking at exogenous estrogens as a risk factor (CB-53884, 74099). B. Seeking differences between pre- and postmenopausal breast cancer (CB-63996, 63998). C. Determining endocrine events at the time of first pregnancy in young and older women (CB-74101). D. Searching for possible relationships between breast cancer and thyroid diseases (CB-84230). E. Examining epidemiologic risk factors in relation to steroid hormone receptors and of hormone binding globulin (CA13556).

Interaction with pathology involves an exploration of possible differences in the epidemiology of different histopathological subcategories of breast cancer (CB-53968, 63997).

Program interest in the natural history of breast cancer led to the examination of the epidemiology of benign breast disease(s), (CB-74102, 74202, 84231, 84319, CA26021, CA13556) and of the minimal disease (CB-74103). The attempt was made to elucidate a mathematical model for the development of breast cancer that fits available incidence data (CB-74100).

Concern has continued for environmental risk factors, not only exogenous steroid hormones, but also other possible carcinogens, in particular the role of diet (CB-53883, 53884, 74104, 84229). Special emphasis on cholesterol and lipids was timely in terms of present interest in a possible negative correlation between risk for cardiovascular disease and risk for cancer (CB-84238, 84318). The principal finding in terminated contract CB-84238 was that there was no relationship between the result of a single serum cholesterol determination and the subsequent development of breast cancer in women. There was, likewise, no direct relationship between total serum lipids nor b-lipoprotein, which is a component of cholesterol, and breast cancer in this cohort. Other results from this study will be reported in a paper to be published in 1981.

Experimental Biology Program: The program of this Section continued to have the largest number (21) of research-oriented contracts however in FY 1980 fifteen of these were terminated (CB-74118, 33883, 74105, 74091, 74189, 74089, 63986, 63983, 74092, 74207, 74090, 53903, 63981, 74108, 74109). Only nine contracts were funded during the year, three of which were service type projects. With the increased emphasis on investigator-initiated research it was not unexpected that this Section of the Branch should receive the largest number of funded grants. In FY 1980 there were 20 grants, competitive and non-competitive in the program that included, one Program Project grant, one New Investigator Award, and one Conference grant.

The categories of basic research (list appends this report) receiving emphasis in the program are the same as those established in the early 1970s. The objective of the grants funded in the past 18 months are within the same categories, and in fact, six of the grantees were originally principal investigators on contracts for this program.

Because of the number of grants and contracts in the program, they will not be discussed individually or collectively in this report. A research summary on each contract that includes information on goals, approaches and progress appends this report. The most impressive progress in the program on experimental biology of the past year has been that made in the culturing of human breast epithelium. The results of the work are important not only to this program but also to diagnosis and epidemiology, carcinogenesis and to preclinical evaluation of therapeutic agents. During the Workshop on Culturing Human Mammary Epithelial Cells it became evident that culturing of the primary cells is a reality and characterization of cells of the gland had made desirable progress. Definition of the nutritional requirements of the cells in vitro has likewise progressed however more work is needed to define the complete requirements. Although progress has been made on primary culturing of mammary epithelium, longterm culturing continues as a major problem.

Therapeutics Program: Fifteen contracts and seven grants were grouped into five major research areas according to their objectives (List appends this report). In several instances a single project fits into more than one area. Additionally, a resource contract (CB-74140) serves as a data repository and as a coordinator for a number of the contract projects as well as for investigators supplying similar data on a voluntary basis. Four contracts were terminated in FY 1980 (CB-74110, 74083, 74205, 74203); six of the contracts were continued only for patient follow-up (CB-43900, 74204, 43917, 43990, 33899, 53917).

Studies categorized under prognosis for clinical tumor recurrence have attempted to identify the patients who are at high risk following treatment of breast cancer clinically confined to the breast or to the breast and regional lymph nodes (CB-74110, 43900, 74205, CA24192). Dr. Wilkinson (CB-74110) reported at the Workshop on Minimal Breast Cancer that his results indicated that the identification of microfoci of tumor by histological examination did not significantly alter the prognosis, compared with the finding of no tumor deposits on routine examination. Under contract CB-43900 an attempt is being made to correlate the sulfotransferase and estrogen binding activities in breast cancer with pathological staging and risk of clinical relapse.

Contract CB-74205 has the task of studying biochemical mechanisms of endocrine-induced breast cancer regression and also, correlating the steroid receptor assay with the clinical course of the disease. These investigators were the first to report that patients with negative ER tumors tend to show earlier recurrence than those whose tumors are ER positive, regardless of tumor size, nodal status or age. The one grant in this category studies the effect of polypeptide factors in cultures of human breast cancer cells and on rat tumors in vivo.

Four contracts were awarded for an RFP on methods for the prediction of response to chemotherapeutic agents (CB-74083, 74205, 74203, 74204). They involved studies of: 1) labeling index, DNA synthesis time, and tumor growth fraction estimation in the unperturbed state and following drug administration; 2) comarison of the effect of drug addition on the labeling index in vitro with the in vivo effects on a rat mammary tumor; 3) attempts to establish multicellular spheroid (MTS) growth in vitro from animal and human breast cancer material and to compare the sensitivity of the MTS to antitumor agents with that of in vivo tumors; 4) a battery of selected enzyme an primary and metastatic breast cancer specimens, and development of mathematical models. While none of the projects were totally successful, useful data were generated and published.

The goal of the studies on prediction of response to endocrine treatment has been to enhance the selection of treatment for individual patients. The relationship between the presence of estrogen receptor protein in breast cancer tissue and the tumors' response to endocrine therapy has been established, although it is not an "all or none phenomenon". Other steroid and polypeptide hormone receptors are being studied for possible additive predictiveness. Also extensive work is underway to develop simplified methods for receptor assay. Three contracts (CB-43963, 43900, 74204), and one grant (CA25586) are funded for these studies. The results of Drs. Jensen and McGuire are well-known in the estrogen binding protein work. Concerning methodology the latest results from Dr. Jensen are: he has developed monoclonal lines of anti-ER-producing hybridoma cells; and, a single rapid ER procedure using controlled pore glass beads. The one grantee intends to show if information about the unchanged nuclear ER and the nuclear Type II binding sites will provide insight into regulatory processes or improve the accuracy of prediction of tumor response to endocrine treatments.

Five contracts (CB-43197, 43990, 33899, 53917, 53851) are categorized as clinical investigations. These projects concern comparisons of treatment regimens and their effectiveness in controlling human breast cancer. Four have been involved with systemic therapy, adjuvant to local therapy, for patients with tumor localized to the breast and axillary nodes. The fifth has tested endocrine suppressive therapy against advanced breast cancer. These studies also were designed to yield information about the biology of breast cancer. The focus of these projects was: 1. CB-43917-Adjuvant regimens were CMF, CMF plus BCG, CMF plus BCG plus killed tumor cell vaccine. Tumor cell vaccine was found to be impractical. Followup has been almost 100 percent and the longterm information is expected to be valuable. 2. CB-43990-Adjuvants are CMF, CMF plus Tamoxifen, CMF plus Tamoxifen plus BCG. Strong features of the study are stratification of patients according to the ER

values and the concurrent followup of 500 patients with Stage I cancer whose ER values are known and who receive no systemic adjunctive treatment. 3. CB-33899-Treatments were L-Pam, cytoxan (C) plus 5-FU plus prednisone, CFP plus postmastectomy radiotherapy. Radiation plus chemotherapy (CFP) did not significantly increase myelosuppression or other toxicity. 4. (CB-53917, L-Pam, CFP, CFP plus BCG. Immunological evaluations with phytohemagglutination analyses and skin tests have not revealed any significant trends among the treatment groups. 5. CB-53851-In patients with inoperable breast carcinoma, the effects are compared of the adrenal suppressive agent aminoglutethimide (AG), surgical adrenalectomy, and antiestrogen therapy. Extensive endocrine studies evaluate the mechanisms of action of AG. Adrenal function recovered about 72 hours after AG treatment was stopped. Tumor regression rates with AG are similar to those with adrenalectomy.

The final category of mechanism of treatment action has two contracts, the one mentioned immediately above (CB-53851) and CB-84221, and three grants (CA26004, 26452, 26287). This group of projects have and are emphasizing the specific tumor, host, or treatment factors through which tumor regression may be achieved. Under contract CB-48221 premenopausal patients receive CMF adjunctive to mastectomy. ER assays are done on their primary tumors and detailed endocrine profiles are obtained prior to therapy and during its course. Patients are followed for evidence of tumor recurrence. Analyses of ER data, endocrine changes, and antitumor effect with attempt to determine if the prolongation of the clinically disease-free interval produced by adjuvant chemotherapy is a result of direct anticancer action of CMF, indirect action via endocrine organ changes or a combination of the two modes of action. Two of the grants (CA26004 and 26287) utilize animal hosts and the other (CA26452) is a human study. The first, a mouse mammary tumor model, is measuring response to C parvum and cytoxan therapy to correlate with tumor kinetics and measurements of selected host immune response, lymph node and spleen cells, bone marrow cells, cultured macrophages and peripheral cells. The second seeks to compare canine and human cancers in regard to structural, immunological and biological characteristics, and to determine whether therapeutic induction of glycoprotein 55 and/or breast cancer specific cell-mediated immunity in dogs with spontaneous breast cancer, influence the biological behavior of the lesions. The third grant has the purpose to determine if endocrine or chemotherapy induce changes in the degree of differentiation of human breast cancer as judged by the concentration of estrogen and progesterone receptors, and if the responses to various therapies can be correlated with the concentrations of the appropriate receptors.

Information Program: The responsibilities of the staff are: 1) The monthly production of the publication Intercom which provides up-to-date listings of scientific papers on breast cancer research in biology, epidemiology, diagnosis, and treatment by title, author and journal reference; a list of meetings and conferences related to the disease; and, abstracts of presentations made at Workshops sponsored by Program. The publication is sent to investigators and institutions throughout the world, approximately 1700 copies are distributed monthly. In FY 1980 the list of those receiving Intercom was checked carefully in order to delete nonresponsive persons thereby diminishing costs. 2) A monthly publication of Intracom which is similar to the listing of the scientific literature found in Intercom, however,

it provides abstracts or summaries of the published literature as well as those on grant and contract activities. The publication is provided only to members of the Staff of the Branch and to the Chairman of the BCTF Committee. 3) Responding to inquiries for literature searches by subject matter and by investigator. The resulting information is provided to staff, both of the Branch and to others in NCI, and to members of the Committee as well as to grantees or contractors.

In FY 1980 the minicomputer programming was completed which permits the 990 system to communicate with the WYLBUR system in the Division of Computer Research and Technology (DCRT). In FY 1979 another software system was developed for communication with the MEDLARS system at the National Library of Medicine, in conjunction with the new software system, the minicomputer has established a communication link for bidirectional transfer of data between the two systems. Thereby the resources of DCRT became available for processing MEDLARS output, including the production of microfiche.

Intracom production was shifted to DCRT facilities by writing an integrated software system that minimizes paper handling and requires a minimum of operator intervention. In addition to hard copy, an output type is produced for conversion to microfiche however DCRT cannot produce the format needed for Intracom at the present time.

Search requests were increased moderately as have the sophistication of the queries. New software permits formatting of search output into clear form suitable for outside distribution. Some of the clerical personnel were trained to carry out this procedure.

Clerical application of ADP technology was augmented by the program written to compose letters automatically for better quality printing on the CT 45 terminal. This reduces clerical time when letters are sent to a list of addresses, such as, those to Committee members, grantees, and contractors.

Training of clerical personnel to use the WYLBUR text editor was begun and with success.

In the coming year Intracom format will be perfected by the addition of indices, search service that will permit remote users with terminals to submit queries for the MEDLARS system and obtain output quickly, and training of personnel in the use of ADP will continue.

BREAST CANCER TASK FORCE GRANT SUPPORTED STUDIES AND ONGOING CONTRACTS

DIAGNOSIS

I. Biophysical Tools for Detection and Diagnosis

A. CA25836-01
Rational Design of Breast Tumor Localizing Agents
Katzenellenbogan, John A.
University of Illinois, Urbana-Champaign

*NO1-CB-84273 Goldberg, Barry
Jefferson Medical College, Philadelphia, PA

NO1-CB-43869 Haberman, JoAnn
University of Oklahoma, Norman

II. Immunodiagnosis

A. CA25653-01
Antigen-Antibody Complexes in Breast Cancer
Chu, Tsann M.
Roswell Park Memorial Institute, Buffalo, NY

B. CA19083-04A1
T-Antigen in Human Cancer Detection
Springer, Georg F.
Evanston Hospital, IL

NO1-CB-74087 Cannon, Grace B.
Litton Bionetics, Kensington, MD

NO1-CB-84228 Cunningham-Rundles, Susanna
Sloan Kettering, New York, NY

NO1-CB-84224 Medof, M. Edward
University of Chicago, IL

III. Biologic Markers in Breast Cancer and Preneoplasia

A. CA25574-01
Isozymes Specific to Human Breast Neoplasia
Yang, Ning-Sun
Michigan Cancer Foundation, Detroit

NO1-CB-84222 Balinsky, Doris
Iowa State University, Ames

*For all contracts, see respective contract summary.

NO1-CB-84316 Jensen, Hanne
University of California, Berkeley

NO1-CB-84223 McCarty, Kenneth S., Jr.
Duke University, Durham, NC

NO1-CB-74206 Schwartz, Morton K.
Memorial Hospital, New York, NY

NO1-CB-53853 Schwartz, Morton K.
Sloan-Kettering, New York, NY

NO1-CB-74086 Sussman, Howard H.
Stanford University, CA

IV. Morphologic Discriminants

A. CA26406-01
In Vitro Studies on Mammary Neoplastic Progression
Asch, Bonnie B.
Beth Israel Hospital, Boston

B. CA28128-01
Tissue Blood Group Antigens and Carcinogenesis
Howard, Donald B.
University of Vermont, Burlington

NO1-CB-74098 Page, David L.
Vanderbilt University, Nashville, TN

NO1-CB-74097 Rosen, Paul P.
Memorial Hospital, New York, NY

V. Resource Contracts (Blood for Marker Studies)

NO1-CB-74213 Bowman, Harold E.
Butterworth Hospital, Grand Rapids, MI

NO1-CB-74138 Rodes, Ned D.
Cancer Research Center, Columbia, MO

NO1-CB-84296 Schutt, Alan J.
Mayo Foundation, Rochester, MN

NO1-CB-74137 Whitney, Leslie W.
Wilmington Medical Center, DL

EPIDEMIOLOGY

I. Genetic Aspects of Susceptibility to Breast Cancer

A. CA27632-01
Genetic Epidemiology of Breast Cancer in Families
King, Mary Claire
University of California, Berkeley

NO1-CB-84274 King, Mary Claire
University of California, Berkeley

II. Steroid Hormones (Endogenous and Exogenous) and Their Contribution to Breast Cancer Risk; Hormonal Aspects of First Pregnancy; Epidemiologic Risk Factors in Relation to Levels of Steroid Hormone Receptors and of Estrogen-Binding β-Globulin

A. CA13556-09
Epidemiology and Natural History of Breast Cancer
Petrakis, Nicholas L.
University of California, San Francisco

NO1-CB-63996 Deubner, David C.
Duke University, Durham, NC

NO1-CB-53884 Nomura, Abraham, M. Y.
University of Hawaii, Honolulu

NO1-CB-63998 Paffenbarger, Ralph S.
University of California, Berkeley

NO1-CB-74101 Preedy, John R. K.
Emory University, Atlanta, GA

NO1-CB-74099 Slone, Dennis: Shapiro, Samuel
Boston University, MA

III. Thyroid Function in Relation to Breast Cancer Risk

NO1-CB-84230 Maloof, Farahe
Massachusetts General Hospital, Boston

IV. Epidemiology of Histopathologic Subcategories of Breast Cancer

NO1-CB-63997 Rosen, Paul P.
Memorial Sloan-Kettering, New York, NY

NO1-CB-53968 Stenkvist, Bjorn
University Hospital of Uppsala, Sweden

V. Epidemiology of Benign Breast Disease; Relation to Epidemiology and Natural History of Breast Cancer; Relation to Ethnicity and Risk of Breast Cancer

A. CA26021-01
Fibrocystic Breast Disease: Epidemiology and Histology
Kelsey, Jennifer L.
Yale University, New Haven, CT

B. CA13556-09
Listed under II
Petrakis, Nicholas L.
University of California, San Francisco

NO1-CB-84231 Bartow, Sue Amelia
University of New Mexico, Albuquerque

NO1-CB-74102 Modan, Baruch
Chaim Sheba Medical Center, Israel

NO1-CB-84319 Petrakis, Nicholas L.
University of California, San Francisco

NO1-CB-74202 Spivey, Gary
University of California, Los Angeles

VI. Epidemiology of Minimal Breast Cancer

NO1-CB-74103 Pasternack, Bernard S.
New York University Medical Center, New York, NY

VII. Environmental Risk Factors for Breast Cancer (beside exogenous hormones)

1. Possible Carcinogens and Mutagens in Breast Fluids

A. CA13556-09
Listed under II
Petrakis, Nicholas L.
University of California, San Francisco

2. Diet (general; lipids and cholesterol; in relation to intestinal flora; in relation to hormone metabolism

NO1-CB-84238 Friedman, Gary D.
Kaiser Foundation Research Institute, CA

NO1-CB-74104 Gorbach, Sherwood
New England Medical Center, Boston

NO1-CB-84317 Higgins, Millicent W.
University of Michigan, Ann Arbor

NO1-CB-84229 Kirschner, Marvin A.
Newark Beth Israel Hospital, NJ

NO1-CB-53883 Modan, Baruch
Chaim Sheba Medical Center, Israel

NO1-CB-53884 Nomura, Abraham
University of Hawaii, Honolulu

NO1-CB-84318 Papatestas, Angelos E.
Mount Sinai School of Medicine, New York, NY

VIII. Mathematical Models for Multifactorial Nature of Breast Cancer, Tested Against Actual Epidemiologic Data

NO1-CB-74100 Lee, John A. H.
Moolgavkar, Suresh H.
Fred Hutchinson Cancer Research Center, Seattle, WA

EXPERIMENTAL BIOLOGY

I. Factors in the Induction of Mammary Tumors

A. CA25915-01
The Radiobiology of Mouse Breast Preneoplasia
Cardiff, Robert D.
University of California,Davis

B. CA27026-01
Basis for Mammary Gland Susceptibility to Carcinogenesis
Russo, Jose
Michigan Cancer Foundation, Detroit

C. CA28646-01
Radioprotective Effect of Mammary Tumor Cell Grafts
Scarantino, Charles W.
University of South Alabama, Mobile

D. CA18664-04
Role of Prolactin in Mouse Mammary Tumorigenesis
Sinha, Yagya N.
Scripps Clinic and Research Foundation,La Jolla, CA

E. CA27293-01
Hormone Influences During Mammary Tumorigenesis
Socher, Susan H.
Baylor College of Medicine, Houston, TX

F. CA20764-04
Neoplastic Mammary Gland: Structure/Function
Strum, Judy M.
University of Maryland, Baltimore

NO1-CB-84227 El-hawari, A. Monaem
Midwest Research Institute, Kansas City, MO

NO1-CB-74118 Frensdorff, Asher
Tel Aviv University, Israel

NO1-CB-84226 Hart, Ronald W.
Ohio State University, Columbus

II. Growth Passage and Characterization of Normal Mammary Epithelial Cells

A. CA05388-21
Biology of Mammary Neoplasia
Nandi, Satyabrata
University of California,Berkeley

B. CA24844-01A1
Characterization of Human Mammary Cells
Stampfer, Martha R.
University of California,Berkeley

NO1-CB-74094 Misfeldt, Dayton S.
Stanford University, CA

NO1-CB-74188 Sato, Gordon
University of California-San Diego

III. Mammary Tumor Response to and Dependence on Hormones

A. CA21606-04
Role of Estrogen and Other Hormones in Breast Cancer
Butler, W. Barkley
Michigan Cancer Foundation, Detroit

B. CA20605-04
Mammary Cancer and Hormone Induced Responses
Clark, James H.
Baylor College of Medicine, Houston, TX

C. CA24512-03
Steroid Hormones in Breast Cancer
Hochberg, Richard B.
St. Luke's-Roosevelt Hospital Center, New York, NY

D. CA26869-01
Nuclear Estrogen Receptors in Breast Cancer
Horwitz, Kathryn B.
University of Colorado Medical Center, Denver

E. CA18664-04
Listed Under I
Sinha, Y. N.
Scripps Clinic and Research Foundation, La Jolla,CA

F. CA27293-01
Listed Under I
Socher, Susan H.
Baylor College of Medicine, Houston, TX

G. CA28698-01
Perinatal Estradiol Influence on Mammary Development
Warner, Marlene H.
Baylor College of Medicine, Houston, TX

H. CA28645-01
Vitamin B6 and Hormone Action in Uteri and Breast Cancer
Wotiz, Herbert H.
Boston University, MA

NO1-CB-33883 Kratochwil,,Klaus
Institute fur Molecularbiologie, Salsburg, Austria

NO1-CB-74189 Rosen, Jeffrey M.
Baylor College of Medicine, Houston, TX

NO1-CB-74105 Sinha, Dilip K.
Roswell Park Memorial Institute, Buffalo, NY

NO1-CB-74091 Talamantes, Frank J.
University of California,Santa Cruz

IV. Interaction of Neoplastic Mammary Cells with Other Cell Populations

A. CA20122-03
Epithelial-Stromal Interactions of Breast Carcinoma
Armstrong, Rosa M.
University of California,San Francisco

B. CA28366-01
Natural Site Preference in Mammary Cancer Biology
Miller, Fred B.
Michigan Cancer Foundation, Detroit

C. CA25179-01
The Fibrotic Response to Human Breast Carcinoma
Stern, Robert
University of California-San Francisco

D. CA20764-04
Listed Under I
Strum, Judy M.
University of Maryland, Baltimore

NO1-CB-63986 Hosick, Howard L.
Washington State University, Pullman

NO1-CB-74089 Lindquist, Richard R.
University of Connecticut, Farmington

NO1-CB-63983 Soloff, Melvyn S.
Medical College of Ohio, Toledo

NO1-CB-84239 Spring-Mills, Elinor
Research Foundation-SUNY, Syracuse, NY

V. Cell Surface Proteins in Relation to Metastasis

A. CA19814-04
Metabolic Fate of Mammary Cell Surface Glycoconjugates
Bernaki, Ralph J.
Roswell Park Memorial Institute, Buffalo, NY

B. CA27909-01
Adhesive Interactions in Mammary Tumor Epithelial Cells
Buck, Clayton A.
The Wistar Institute, Philadelphia, PA

C. CA25173-01A1
Sialoglycoproteins of a Metastatic Mammary Tumor
Carraway, Kermit L.
Oklahoma State University, Stillwater

D. CA19455-04
Use of Membranes to Assess Phenotypic Expression
Ceriani, Roberto L.
Children's Hospital Center, Oakland, CA

E. CA08418-14
Glycoproteins from Cancer Cells
Codington, John F.
Massachusetts General Hospital, Boston

F. CA27314-01
Plasma Membrane and Metastasis in Rat Mammary Cancer
Fairbanks, Grant
Worcester Foundation for Experimental Biology, Shrewsbury, MA

G. CA25418-01A1
Murine Virus Cross-Reaching Antigen in Human Tissue
Hackett, Adeline J.
University of California, Berkeley

H. CA26825-01
Antigen Specificity NK Activity and Suppression in MMTV
Lane, Mary-Ann
Sidney Farber Cancer Institute, Boston, MA

I. CA28844-01
Mammary Cancer Metastasis
Nicholson, Garth L.
University of Texas System Cancer Center, Houston,TX

NO1-CB-74092 Neidermeier, William
University of Alabama, Birmingham

VI. Prevention of Neoplastic Transformation

A. CA25973-01
Cancer Therapy with Enterovirus Plus Cyclophosphamide
Biggs, Max W.
University of California, Livermore

NO1-CB-74207 Moon, Richard D.
ITT Research Institute, Chicago, IL

NO1-CB-74090 Welsch, Clifford W.
Michigan State University, East Lansing

VII. Pathophysiology of the Solid Mammary Carcinoma and its Relation to Matastasis

A. CA28735-01
Proteoglycans and Basal Lamina Structure and Function
Bernfield, Merton R.
Stanford University, CA

B. CA25500-01
Osteotropism of Mammary Carcinoma Metastasis
Mundy, Gregory R.
University of Connecticut, Farmington

C. CA05388-21
Listed Under II
Nandi, S.
University of California, Berkeley

NO1-CB-53903 Bernfield, Merton R.
Stanford University, CA

NO1-CB-63981 Gross, Joseph F
University of Arizona, Tucson

NO1-CB-74108 Leboy, Phoebe S.
University of Pennsylvania, Philadelphia

NO1-CB-74109 Wirl, Gerhard
Institute for Molecularbiologie, Salsburg, Austria

VIII. Service, Support and Resource

A. CA28816-01
Meeting-Mammary Cancer in Experimental Animals and Man
Hageman, Philomena C.
Netherlands Cancer Institute, Amsterdam

NO1-CB-74175 Bogden, Arthur C.
Mason Research Institute, Worcester, MA

NO1-CB-84225 Furthmayr, Heinz
Yale University, New Haven, CT

NO1-CB-84314 Goldberg, Burton
New York University, New York, NY

TREATMENT

I. Prognosis for Clinical Tumor Recurrence

A. CA24129-01A1
Control of Breast Cancer by Serum Factors
Osborne, C. Kent
University of Texas Health Science Center, San Antonio

B. CA26935
CEA and FHAP as Breast Cancer Management Parameters
Tormey, Douglass C.
University of Wisconsin, Madison

NO1-CB-43900 Dao, Thomas L.
Roswell Park Memorial Institute, Buffalo, NY

NO1-CB-23862 McGuire, William L.
University of Texas Health Science Center, San Antonio

NO1-CB-74110 Wilkinson, Edward J.
Medical College of Wisconsin, Milwaukee

II. Prediction of Response to Chemotherapeutic Agents

NO1-CB-74204 Hilf, Russell
University of Rochester, NY

NO1-CB-74083 Schiffer, Lewis M.
Allegheny-Singer Research Corp., Pittsburgh, PA

NO1-CB-74205 Von Hoff, Daniel D.
University of Texas Health Science Center, San Antonio

NO1-CB-74203 Yuhas, John M.
University of New Mexico, Albuquerque

III. Prediction of Response to Endocrine Therapy

A. CA27470-01
Receptor Studies in Human Breast Cancer
Hollander, Vincent P.
Hospital for Joint Diseases, New York, NY

B. CA25586-01A1
Studies of Anamalous Receptors in Breast Cancer
Panko, Walter B.
Baylor College of Medicine, Houston, TX

NO1-CB-43900 Dao, Thomas L.
Roswell Park Memorial Institute, Buffalo, NY

NO1-CB-43963 Jensen, Elwood V.
University of Chicago, IL

IV. Clinical Investigations

NO1-CB-33899 Ahmann, David L.
Mayo Foundation, Rochester, MN

NO1-CB-43990 Hubay, Charles A.
Case Western Reserve University, Cleveland, OH

NO1-CB-43917 Morton, Donald L.
University of California, Los Angeles

NO1-CB-53851 Santen, Richard J.
Pennsylvania State University, Hershey

NO1-CB-53917 Scanlon, Edward F.
Evanston Hospital, IL

V. Mechanisms of Treatment Action

A. CA26004-01
Therapy Effects on Tumor Kinetics and Immune Parameters
Fisher, Bernard
University of Pittsburgh, PA

B. CA26287-01
Canine Model for Human Breast Cancer Immunotherapy
Hurvitz, Arthur I.
Animal Medical Center, New York, NY

C. CA26452-01
Therapy-Induced Changes in Breast Cancer
Panko, Walter B.
Baylor College of Medicine, Houston, TX

NO1-CB-84221 Das Gupta, Tapas K.
University of Illinois, Chicago

NO1-CB-53851 Santen, Richard J.
Pennsylvania State University, Hershey

VI. Data Analysis Resource

NO1-CB-74140 Dunsmore, Marlene
Mason Research Institute, Rockville, MD

CONTRACT RESEARCH SUMMARY

Title: Biological Markers in Breast Cancer

Principal Investigator: Dr. Ned D. Rodes
Performing Organization: Cancer Research Center
City and State: Columbia, MO

Contract Number: NO1-CB-74138
Starting Date: 9/1/77 Expiration Date: 8/31/80

Goal: To serve as a Breast Cancer Task Force specimen resource for blood from breast cancer patients and controls to be utilized in search for and verification of breast cancer markers.

Approach: 30 ml of blood will be collected from volunteer Breast Cancer Detection Demonstration Project and Women's Cancer Control Program screenees after they have signed appropriate consent forms. In the event of subsequent breast biopsies upon any of the participants, a 30 ml postoperative specimen will also be collected from postmastectomy cases within three months. In addition, prebiopsy and postmastectomy blood samples will be obtained from hospitalized ladies in the Columbia area who agree to participate in the program. Emphasis will be placed upon collection from abnormal hospitalized volunteers. No special requirements will be placed upon the participants in terms of diet or liquid intake. Anniversary serum from malignant patients will be obtained for three, possibly five years. All blood will be stored at -70^{o} centigrade, and then shipped to NCI-designated blood bank facility with appropriate clinical data.

Progress: In the 29 months of serum collection (from 12/77 to 4/80), 9,696 individual samples of blood were fully processed; this number of blood samples yielded 104,818 vials. The average serum collection per participant was 11.8 ml. The total number of "voids" (no draws; broken clot tubes; collapsed veins; hemolysis, etc.) was limited to 396 over the entire 29 month collection period. Therefore, blood samples plus "voids" equals 10,092 total participants. The average acceptability for research of shipments during this time frame was 96.1%.

Since September 1979, special attention has been paid to the collection of blood samples from prebiopsy/postbiopsy/postmastectomy ladies. In the 8 months (from 9/79 until 5/80) of specimen collection from this special group of ladies, 84 specimens have been collected from 67 participants, 24 of which proved to have breast cancer. Specimens were collected from an additional 27 ladies who were referred to the Center because of symtomatic breast disease but who were not later subjected to biopsy. The guidelines for future workscope point toward an increased emphasis on specimen collection from this biopsy/mastectomy/benign-breast-disease group.

Project Officer: Ihor Masnyk, Ph.D., Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $50,061

CONTRACT RESEARCH SUMMARY

Title: Biologic Characterizations of "Premalignant" Human Mammary Epithelial Hyperplasias

Principal Investigator: Dr. Kenneth Scott McCarty, Jr.
Performing Organization: Duke University
City and State: Durham, NC

Contract Number: NO1-CB-84223
Starting Date: 8/1/78 Expiration Date: 7/31/81

Goal: To characterize potentially "premalignant" epithelial lesions of the human breast using biochemical, morphologic and immunologic techniques.

Approach: Analyses are performed on lesions observed in subcutaneous mastectomy specimens, (mastectomy performed for indications other than malignancy); benign biopsies (biopsy performed for suspected malignancy); and samples obtained from non-carcinomatous areas of mastectomy specimens (mastectomy performed for overt malignancy). These are compared to carcinomatous areas of the breast cancer specimens. Lesions are classified by subgross, light, and electron microscopic techniques as appropriate. The presence of measurable steroid receptors, cyst fluid derived estrogen-progesterone binding protein, and endogenous peroxidase are compared to the presence or absence of K-casein, and CEA. Immunologic characterizations are approached by the assessment of IgG, IgA, and IgM localization with characterization of eluted immunoglobulin in selected cases. The expression of ABH blood group antigens and/or the unmasking of component antigens, are assessed by immunohistology.

Progress: A statistically significant association of the simultaneous presence of certain patterns of intraacinar epithelial hyperplasias and carcinoma has been confirmed. Apocrine and adenosquamous differentiation have been characterized ultrastructurally. Attenuation of the epithelial stromal junction was associated with IgG localization and alteration in the ABH blood group antigen expression in mammary dysplasias. CEA is seen in malignant lesions and severe epithelial dysplasias but only rarely (<5%) in the simple fibrocystic lesions. K-Casein does not appear to correlate eith E_2 receptor. The localization of immunoglobulin G in a pericellular pattern has been observed in dysplastic epithelial hyperplastic lesions (usually type 14 positive), while apical IgA localization has characterized the non-dysplastic benign lesions studied. IgG localization correlates with lymphopenia. FITC labelled steroids to localize sex steroid binding has suggested that 17 substituted compounds correlate best with SDGA techniques, although E_2-FITC binding to low-affinity-high-capacity proteins is likely due to the correlation of binding to the presence of estrogen-progesterone binding protein. Menstrual cycle fluctuations of estrogen and progesterone receptor have been observed in benign, dysplastic and malignant lesions. The levels of intermediate affinity estrogen-progesterone binding protein are maximal in the late luteal phase.

Project Officer: D. Jane Taylor, Ph.D.
Program: Breast Cancer Diagnosis
FY 80 Funds: $79,675

CONTRACT RESEARCH SUMMARY

Title: Isoproteins in Normal, Benign and Malignant Breast Tissues

Principal Investigator: Dr. Doris Balinsky
Performing Organization: Iowa State University
City and State: Ames, IA

Contract Number: NO1-CB-84222
Starting Date: 8/1/78 Expiration Date: 7/31/81

Goal: To detect, quantitate, and characterize various isozymes or isoproteins in normal, benign and neoplastic breast tissue in an attempt to identify "tumor markers" which may have significance in the early detection of breast cancer or identification of patients with occult dissemination after local treatment.

Approach: A number of enzymes known to have differing isozyme patterns in different tissues will be assayed in human normal, benign, malignant and metastatic breast tissues. Isozyme patterns of those enzymes having detectable activity will be examined by electrophoresis on polyacrylamide gel and cellulose acetate. If interesting isozyme differences are found between normal and cancerous tissues, the isozymes will be purified and characterized. The data will be correlated with data on estrogen and progesterone receptors on the same material and with clinical and histopathologic data.

Progress: The isozyme patterns of twenty-three different enzymes have been examined to date by cellulose acetate electrophoresis. The patterns were compared in surgical and autopsy breast tissue from normal patients (N), in "pure" ductules, in benign human tumors (B), in malignant human (Ca), and rat tumors, and in MCF-7 cells. The proportions of the M(A) subunit of lactate dehydrogenase were MCF-7 > Ca > B > N. Also, the mitochondrial form of malate dehydrogenase was higher in Ca > B > N. For both enzymes the differences between groups were statistically significant. The Ca had the highest activities of these enzymes and of other glycolytic enzymes; B had intermediate activities, and N had the lowest. Acetylcholinesterase A_4 stained heavily in 80% of Ca, 50% of B and 12% of N. The A_5 isozyme was a major band in 50% of the Ca, but in none of the B and N tissues. Butyrylcholinesterase B_3 was the major band in 50% of Ca and in almost all the B and N tissues, whereas the B_2 isozyme stained heavily in 40% of Ca, 90% of B and only 25% of N. MCF-7 cells had A_5 as a major band and some B_3. Further studies on the incidence of the D isozyme and of minor bands are underway.

Project Officer: D. Jane Taylor, Ph.D.
Program: Breast Cancer Diagnosis
FY 80 Funds: $71,200

CONTRACT RESEARCH SUMMARY

Title: The Use of Ultrasound in the Diagnosis of Breast Cancer

Principal Investigator: Dr. Barry Goldberg
Performing Organization: Jefferson Medical College
City and State: Philadelphia, PA

Contract Number: NO1-CB-84273
Starting Date: 5/1/78 Expiration Date: 6/30/80

Goal: To develop characteristic ultrasound patterns of benign and malignant breast pathology in order to evaluate the efficacy of high resolution ultrasonic equipment in the detection of early breast cancers.

Approach: Two different ultrasonic machines, the Octoson B scan and the Johnson and Johnson real time scanner have been clinically evaluated. Patients coming to surgery for suspected breast carcinoma have a preoperative X-ray mammogram, physical examination and ultrasonic examination. There were repeat examinations of any excised breasts. In addition, 8-10 cases per week recommended for biopsy or aspiration of suspected cysts were similarly examined. Two-hundred and fifty asymptomatic patients were also enrolled in the program. Ultrasonic patterns were correlated with histopathology and X-ray mammograms in both in vitro and intact breast tissues. A secondary objective was to advance the state of the art of detection of breast cancer by incorporating the ultrasonic wave form signal analysis and acoustic signaling techniques in a computer learning procedure under a subcontract. The Octoson ultrasound machine was modified to obtain these wave forms.

Progress: One-thousand and six-hundred sixty-four patients have been examined to date under this research protocol. Evaluation of the first 1,029 patients has been completed. There were 258 asymptomatic patients in this group. There were 173 biopsies performed on this population of which there were 79 proven cancers. Fifty-eight of the cancer cases had both the ultrasound examination and X-ray mammography. In this group the sensitivity of ultrasound for breast carcinoma was .69 and X-ray mammography was .74. If the clinical information was included in the ultrasound interpretation the sensitivity rose to .79. The specificity of the ultrasound examination was .79 which rose to .83 using clinical data. The true positive rate for ultrasound was .3 compared to .41 for X-ray mammography. In all cancer cases (79) ultrasound's sensitivity and specificity using the clinical information was .80 and .82 respectively.

Project Officers: D. Jane Taylor, Ph.D., Donald E. Henson, M.D.
Program: Breast Cancer Diagnosis
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunologic Cross-Reactions Between Breast Cancer and Mammary Tumor Virus

Principal Investigator: Dr. Grace B. Cannon
Performing Organization: Litton Bionetics, Inc.
City and State: Kensington, MD

Contract Number: NO1-CB-74087
Starting Date: 7/1/77 Expiration Date: 6/30/80

Goal: To perform the leukocyte migration inhibition (LMI) assay for the detection of cell-mediated immunity (CMI) against mouse mammary tumor virus (MMTV) antigens in order to ascertain the usefulness of the LMI assay for the early diagnosis, monitoring and prognostic predictability of patients with breast cancer.

Approach: Peripheral blood mononuclear cells from breast cancer patients (prior to biopsy and post-mastectomy), benign disease patients (pre- and post-biopsy), patients with other types of cancer, patients with gynecological disease, and healthy female controls will be tested under code in the indirect LMI assay with MMTV and MCF-7.

Progress: We have nearly completed the testing of all categories of patients and control individuals. At the suggestion of the Project Officer and her committee, we have obtained the collaboration of a pathologist, Dr. Sanford Barsky of George Washington University, to re-evaluate the pathology of the lesions of the benign patients. At this time, he has re-examined the pathology slides of 102 benign patients who were previously tested in the direct test and 20 benign patients who were tested in the indirect test. He placed the patients in six different categories: 0 - fat or stroma, occasional cysts; 1 - prominent cysts, apocrine metaplasia, focal adenosis; 2 - prominent sclerosing actenosis, crowding of ducts and acini, prominent apocrine metaplasia but no intraductal proliferation; 3 - intraductal proliferation either in the form of papillomatosis or hyperplasia, not atypical hyperplasia; 4 - intraductal papilloma; 5 - extremely atypical hyperplasia. Using a cut-off based on the reactivity of the pre-op gynecological patients, the following proportions of patients in the respective categories were reactive to MMTV: 0, 0/23; 1, 2/33 (6%); 2, 1/20 (5%); 3, 0/10; 4, 1/5 (20%); 5, 0/1; and to GP-52: 0, 2/18 (11%); 1, 5/29 (17%); 2, 2/18 (11%); 3, 3/12 (25%); 4, 0/3 in the direct test. Dr. Barsky is re-evaluating more patients tested in the indirect test and we are re-evaluating all our results with respect to age of patients and control individuals.

Project Officer: D. Jane Taylor, Ph.D. and Donald E. Henson, M.D.
Program: Breast Cancer Diagnosis
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: NCI Sera Bank Facility for the Breast Cancer Task Force

Principal Investigator: Dr. Allan J. Schutt
Performing Organization: Mayo Clinic/Foundation
City and State: Rochester, MN

Contract Number: N01-CB-84296
Starting Date: 9/1/77 Expiration Date: 8/31/81

Goal: To establish and maintain a storage facility for serum specimens to be used by the Breast Cancer Task Force in a program designed to search for biological markers in breast cancer.

Approach: Sera specimens will be secured from breast cancer patients, benign disease patients, controls, and a screening population under separate contracts setting up a patient resource. The material will be processed, recorded and stored in -70°C deep freezers under easily retrievable conditions with all clinical data available. The sera will be used in the search for and verification of new breast cancer markers.

Progress: Collection and inventory methods have been developed. A special vial is supplied to the collection areas. An operational shipping schedule has been established on a regular basis. Specimens have been catalogued and systematically stored and number-coded. We have continued careful quality control. Methods of assembling number-coded panels of vials have been worked out with E.G.&G. Mason Research Institute. Inventory status of the stored sera specimens collected during the first thirty-one months of the project is listed:

Inventory Status April 1, 1980	No. Patients	No. Vials
Screening Project (Columbia, MO)	9,542	102,014
Operative Patients (Grand Rapids, MI)	1,900	19,808
Operative Patients (Wilmington, DE)	481	4,908
TOTAL:	11,923	126,730

Collected Since Jan. 30, 1980 Report	Patients	No. Vials
Screening Project (Columbia, MO)	1,035	11,191
Operative Patients (Grand Rapids, MI)	259	2,821
Operative Patients (Wilmington, DE)	27	279
TOTAL:	1,321	14,291

Shipping of coded serum panels to breast cancer biological marker investigators has commenced. Three such panels have been shipped to date (582 vials from 330 patients).

Project Officer: Ihor J. Masnyk, Ph.D., Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $51,400

CONTRACT RESEARCH SUMMARY

Title: Prognostic Factors for Disseminated Cancer in Patients with Early Breast Cancer

Principal Investigator: Dr. Paul Peter Rosen
Performing Organization: Memorial Hospital
City and State: New York, NY

Contract Number: NO1-CB-74097
Starting Date: 8/1/77 Expiration Date: 7/31/81

Goal: To determine the frequency of subsequent metastatic breast cancer in a group of females with "early" invasive carcinoma and to identify those clinical and/or pathologic features or groups of features that are indicators of a high or low risk for dissemination of carcinoma.

Approach: A study population of 250 women with primary infiltrating breast carcinomas 1.0-2.0 cm in diameter and a subset of 100 women with malignant tumors 1 cm or less in diameter will be identified in a retrospective study (1964-69), and followed for at least 10 years. These patients will have been treated by at least a modified radical mastectomy and have been found to have negative lymph nodes. The relationships observed between pathologic and clinical data and survival will be evaluated in order to identify those factors associated with a high or low frequency of recurrence. Patients with primary tumors 2 cm or less with positive lymph nodes will also be studied to provide a basis for comparing the distribution of various features.

Progress: All pathology records for the years 1964, 1965, 1966, 1967, 1968 and 1969 (60,617 reports) have been reviewed. Among approximately 1800 primary mammary carcinomas treated in this hospital, we have confirmed 134 cases with a primary lesion 1 cm or less in diameter and 248 cases grouped in the 1.1-2.0 cm category. Follow-up at present reveals that 44 patients have died of breast carcinoma. Analysis of risk factors has thus far revealed that patients with intralymphatic tumor emboli in the breast had a significantly greater likelihood of recurrence than those who did not exhibit this lesion. An additional 192 patients with primary cancers less than 2 cm in diameter have been identified. They constitute 33% of the entire 574 patient population. Recurrence has been documented in 32% of those with a single lymph node metastasis and 23% have died of breast cancer.

Project Officer: D. Jane Taylor, Ph.D., Donald E. Henson, M.D.
Program: Breast Cancer Diagnosis
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Longitudinal Studies of Biologic Markers in Breast Cancer Patients

Principal Investigator:	Dr. Morton K. Schwartz
Performing Organization	Memorial Hospital
City and State:	New York, NY

Contract Number: NO1-CB-74206
Starting Date: 8/1/77 Expiration Date: 7/31/81

Goal: To assay human breast cancer tissue and sera prior to mastectomy and throughout the clinical course of the disease for potential markers, to determine how effective such markers would be for early detection of breast cancer and its recurrence, and to gain an understanding of the relationship between the concentration of the marker substances in the individual tumor and in the serum levels of the host.

Approach: Serum and tissue specimens from patients entering for diagnosis and for primary therapy will be assayed for carcinoembryonic antigen, sialyl-transferase, phosphohexose isomerase, spermine, ferritin, lactic dehydrogenase and its isoenzymes, β-hCG, γ-glutamyltranspeptidase, α-fetoprotein and IgA. In tissue only glucose-6-phosphate dehydrogenase, estrogen and progesterone receptor protein, total protein and DNA will be determined to serve as points of reference. Alkaline phosphatase, calcium and transaminase will be assayed in serum. Sera specimens will be collected prior to surgery, before discharge and then sequentially, every three months the first year, and every six months thereafter. Tissue will be obtained at time of biopsy and mastectomy. Should recurrences follow, metastatic tissue where available will undergo the same testing procedure. Normal breast tissue will be assayed. Tissue and serum biochemical-immunochemical data will be compared and changes and differences related to the clinical status of the patient.

Progress: As of May 1, 1979, 390 patients had been entered into the study. Studies have indicated that there is little variability in the parameters studied in different sections of the same tissue. Cancerous tissues have concentrations in most instances much higher than normal breast tissue and for most constituents the concentrations are on the average more than 3 times the concentrations of normal tissue. The concentrations of some constituents were related to age of the patient and the hormone receptor presence in the tissue. In the first group of patients 72% had at least 1 marker elevated in the blood before surgery and 51% had at least 1 marker elevated 3 months later. The patients are being followed to determine which if any combination of markers can be useful in the management of patients with breast cancer.

Project Officer: D. Jane Taylor, Ph.D.
Program: Breast Cancer Diagnosis
FY 80 Funds: $30,000

CONTRACT RESEARCH SUMMARY

Title: Biological Markers in Breast Cancer: Patient Resource

Principal Investigator: Dr. Harold E. Bowman
Performing Organization: Saint Mary's Hospital
City and State: Grand Rapids, MI

Contract Number: N01-CB-74213
Starting Date: 9/15/77 Expiration Date: 9/14/81

Goal: To develop a Breast Cancer Task Force specimen resource for blood from breast cancer patients, benign disease patients and controls to be used in a search for and verification of new breast cancer markers.

Approach: Thirty milliliters of blood will be collected from breast disease patients who are scheduled to undergo biopsy and/or primary surgery for breast lesions prior to surgery. Another specimen will be collected, when feasible, 5-10 days postmastectomy from the same patient. Annual drawings are made on patients with malignant diagnosis. Patients with benign tumors are requested to complete annual questionnaires for a period of two years after biopsy. Serum specimens will be stored at -70° C, then shipped to an NCI designated blood bank facility with appropriate clinical data.

Progress: The program has been presented to the surgery sections in each participating hospital. Surgeons who perform 95% of all breast biopsies in the participating hospitals have signed letters of agreement allowing their patient to enter directly into the study. Since the inception of the program, 1,514 patients have been entered into the study; 330 of these patients have been found to have malignant breast disease. 20,737 vials containing serum specimens have been shipped to the central storage facilities at Mayo Clinic. 126 annual blood samples have been drawn from participating patients with malignant diagnoses. 236 participating patients with benign tumors have completed the annual questionnaires.

Project Officer: Ihor J. Masnyk, Ph.D., Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $30,700

CONTRACT RESEARCH SUMMARY

Title: Prognostic Significance in Breast Cancer of Regional Lymph Node Immune Response

Principal Investigator: Dr. Susanna Cunningham-Rundles
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: N01-CB-84228
Starting Date: 9/1/78 Expiration Date: 8/31/81

Goal: Evaluation of the relevance of regional lymph node immune response to prognosis in breast cancer. This will require an assessment of cell-mediated immune reactivity in well-standardized in vitro assays to defined antigens associated with the development of disease.

Approach: Immunological procedures will be performed on lymph nodes of 100 patients with primary operable breast cancer, using a total of 4-6 lymph nodes per patient including both negative and positive nodes, ideally from each level (I-III). In addition, control lymph nodes will be obtained from 50 other patients. The assays to be done on both peripheral blood and lymph node mononuclear cells include leukocyte migration inhibition factor (LMIF) and lymphocyte transformation tests, the latter comprising PHA, Candida, E. coli, T-antigen and MuMTV. A third class of newer tests includes natural killer cells, suppressor cells, cell surface markers and monocyte function assays. Clinical and demographic data as well as histopathologic data on tumor size and histologic type will be obtained. Three-year follow-up information on recurrence and survival status will be recorded. Correlation of all factors will be statistically performed.

Progress: The principal findings of this study to date may be briefly summarized as follows: Spontaneous lymphocyte mediated cytotoxicity (SLMC) of peripheral blood lymphocytes (PBL) was found to be normal for most patients and significantly increased SLMC in regional lymph nodes was observed in some patients. Corresponding increase of T γ cells in the regional lymph node cell population was also observed. These findings are in contrast to studies of control lymph node cells where SLMC and T γ cells were virtually absent. Increased peripheral T γ cells were found in 50% of patients studied. Comparison of peripheral blood mononuclear cells and regional lymph node cell responses to mitogens and antigens including T antigen and MuMTV has indicated that significant differences between these responses are characteristic.

Project Officer: D. Jane Taylor, Ph.D., Donald E. Henson, M.D.
Program: Breast Cancer Diagnosis
FY 80 Funds: $114,360

CONTRACT RESEARCH SUMMARY

Title: Biochemical Analysis of Human Breast Cyst Fluid (BCF)

Principal Investigator: Dr. Morton K. Schwartz
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-53853
Starting Date: 6/30/75 Expiration Date: 9/14/81

Goal: To establish the biochemical and immunochemical composition of human BCF; to search for a marker which might be useful in understanding the formation of cysts, their treatment, and the risk of patients with cysts developing cancer.

Approach: BCF from patients with cystic mastopathy will be analyzed for polypeptide and steroid hormones, tumor-associated antigens, enzymes, lipids, minerals and trace elements. These data will be correlated with each other and with the demographic information. Fluid will be analyzed from patients with recurrent cysts and clinical follow-up will be obtained on all patients.

Progress: Patient entry has been completed. There have been 1,044 patients (1,830 specimens) entered into the study. About 25% of the patients have had recurrent cysts and additional specimens submitted for analysis since their initial aspiration. As previously noted, the concentrations in many BCF specimens of CEA, α-fetoprotein, β-subunit hCG, calcium, copper, zinc, total protein, albumin, cholesterol, phosphohexose isomerase, γ-glutamyltranspeptidase, β-glucuronidase, lactic dehydrogenase, amylase, dehydroisoandrosterone, androsterone, and their sulfates are many-fold the concentrations of these constituents found in serum. Correlation of BCF concentrations for many of these constituents in the same patients were found to be significant. The specimen variation was much less in specimens from the same patients obtained either at the same time or on subsequent aspiration than that from patient to patient. Notwithstanding the large BCF to serum gradient, isotope studies have indicated a slow influx and efflux of the steroids into the cyst fluid. The fluid appears to contain material both of a secretory nature and that resulting from cell breakdown. BCF contains low but detectable levels of terminal deoxynucleotidyl transferase (TdT) and DNA polymerases were observed in BCF and in supernatant from cells from BCF maintained in culture media. Biostatistical analysis has indicated that in the 40-65 years age group where the cyst occurrence is most prominent, the incidence of breast cancer in cyst patients is 625 per 100,000 as compared to 144 per 100,000 from estimates in normal women. Follow-up of patients is continuing.

Ref. Bradlow, H.L., Schwartz, M.K., Fleisher, M., et al.: Accumulation of Hormones in Breast Cyst Fluid. J. Clin. Endocrin. Met. 49:778-782, 1979.

Project Officer: D. Jane Taylor, Ph.D., Donald E. Henson, M.D.
Program: Breast Cancer Diagnosis
FY 80 Funds: $9,555

CONTRACT RESEARCH SUMMARY

Title: Longitudinal Studies of Biologic Markers in Breast Cancer Patients

Principal Investigator: Dr. Howard H. Sussman
Performing Organization: Stanford University School of Medicine
City and State: Stanford, CA

Contract Number: NO1-CB-74086
Starting Date: 8/1/77 Expiration Date: 7/31/81

Goal: To assay human breast cancer tissue and sera, prior to mastectomy and throughout the clinical course of the disease, for potential markers to determine how effective such markers would be for early detection of breast cancer and its recurrence, and to gain an understanding of the relationship between the concentration of the marker substances in the individual tumor and in the serum levels of the host.

Approach: Tumor and sera of patients presenting initially for diagnosis and primary therapy (Category 1) will be assayed for the fetoplacental proteins: i.e., placental alkaline phosphatase (PAP), human chorionic gonadotropin (hCG) α and β, and carcinoembryonic antigen (CEA); and tests on sera will be repeated sequentially every three months. Patients developing recurrences (Categories 2 and 3) will be included with a study population from the Northern California Oncology Group. Another objective of the study will be to identify new ectopically synthesized placental membrane proteins, which may be common to both trophoblasts and neoplastic breast tumor cells.

Progress: One hundred patients from Category 1 and 25 patients from Category 3 (recurrent disease) have been signed into the study and are being followed longitudinally throughout the course of the disease for the expression of tumor markers. Seventy-eight patients from Category 3 have been tested prior to therapy for PAP and α-hCG. Serum PAP was elevated (>2 ng/ml) in 20% of the metastatic patients, and serum α-hCG was elevated (>3 ng/ml) in 14%. Fifty-four Category 3 patients were tested for serum β-hCG and 35% were elevated (>2.5 ng/ml) for this marker. In the primary patient group, 5/63 (8%) had elevated initial serum PAP levels, 3/53 (6%) had elevated serum α-hCG levels, and 19/92 (21%) had elevated β-hCG levels. Initial serum values for CEA have been determined for 74 primary breast cancer patients and 10 benign patients. Forty-one percent of the primary patients were elevated in CEA (>2.5 ng/ml). Assay of 78 primary tumors for PAP showed no marked production of PAP. Assay of 11 metastatic tumors from breast adenocarcinoma showed one strong PAP-producing tumor metastatic to bone. The assay of 115 primary tumors for α-hCG gave a higher incidence of elevated values, (21/115 or 18%; >0.5 ng/mg protein). β-hCG was minimally elevated in 1/103 tumors (2%). Assay of 40 metastatic tumors showed 10/40 (25%) of the samples having elevated α-hCG levels (>0.5 ng/mg protein). 1/19 (4%) of the metastatic tumors showed elevated β-hCG levels.

Project Officer: D. Jane Taylor, Ph.D. and Donald E. Henson, M.D.
Program: Breast Cancer Diagnosis
FY 80 Funds: $35,000

CONTRACT RESEARCH SUMMARY

Title: Biologic Characterization of "Premalignant" Human Epithelial Hyperplasias

Principal Investigator: Dr. Hanne M. Jensen
Performing Organization: University of California
City and State: Davis, CA

Contract Number: NO1-CB-84316
Starting Date: 9/18/78 Expiration Date: 9/17/81

Goal: To establish if angiogenesis is a reliable criterion for precancer of the human breast and to characterize by biochemical and immunological markers the type(s) of lesions that possess angiogenic capacity from those that do not.

Approach: Fresh, parenchymal structures and hyperplastic lesions stained with Methylene Blue Chloride are isolated and studied for angiogenic potential by transplantation onto the iris of female rabbits. 6μ thick frozen sections of parts of isolated structures and lesions are stained with fluorescent labeled anti-human immunoglobulins G, A and M, to characterize them immunologically and with fluorescent labeled estrogen and progesterone to detect hormone receptors.

Progress: From 34 benign biopsies, 9 cancerous biopsies, 6 mastectomies for cancer, 4 subcutaneous mastectomies for high cancer risk, 6 reduction mammoplasties and 2 males with gynecomastia, 824 transplants were made. They were derived from 592 individual structures and lesions. Angiogenesis is possible if transplants remain fixed to the iris and contain epithelium. Transplants from 408 lesions were successful. 31 angiogenic lesions were derived from cancer-associated breasts; 48 from non-cancerous cases. Of 329 non-angiogenic lesions, 303 were from non-cancerous cases. Papillomas are angiogenic like carcinomas. 32 atypical lobules were studied. 12 of 16 (75%) from cancer-associated breasts were angiogenic. 5 of 16 (31%) from non-cancerous breasts were angiogenic. Angiogenic atypical lobules tend to be bigger, to have prominent lymphatic network, to secrete immunoglobulin A and to display succulent stroma. Adenoma, developing lobules and lactating lobules commonly are angiogenic. Fibroadenomas, apocrine cysts, normal ducts and most normal lobules are non-angiogenic. Ducts do not secrete immunoglobulin, most lobules do.

Since developing and lactating lobules evoke angiogenesis, this biologic behavior may reflect a metabolically active epithelium. The high angiogenic rate among cancer associated atypical lobules and the fact that angiogenic atypical lobules were derived from only 2 of 34 benign biopsies and one woman with a familial breast cancer history suggest that angiogenesis is a marker for later cancerous development. Loose stroma within atypical lobules may be predictive of angiogenesis. The secretion of immunoglobulin by atypical lobules suggests lobular derivation as does their microarchitecture.

Project Officer: D. Jane Taylor, Ph.D., Donald E. Henson, M.D.
Program: Breast Cancer Diagnosis
FY 80 Funds: $91,550

CONTRACT RESEARCH SUMMARY

Title: Detection of Immune Complexes in Sera of Patients with Breast Cancer

Principal Investigator:	Dr. M. Edward Medof
Performing Organization:	The University of Chicago
City and State:	Chicago, IL
Contract Number:	NO1-CB-84224
Starting Date:	9/1/78
Expiration Date:	8/30/81

Goal: To assay sera from patients with breast cancer, benign breast disease, and normal volunteers for immune complexes (ICs) employing multiple techniques for IC detection and quantitation, then to correlate levels where applicable of individual IC subpopulations with presence or absence of tumor, tumor progression, response to therapeutic intervention and clinical course.

Approach: Four detection techniques for ICs (Raji cell receptor assay, conglutinin-binding assay, solid phase C1q binding assay, and PEG-C1q precipitation assay) are established in this laboratory and will be run on sera of 20 new breast cancers, 50-100 benign breast disease patients, and 100-300 patients with no current breast disease as well as 25 to 75 patients with metastatic disease in the second year. Serum samples will be assayed for soluble and insoluble, complement bearing and IgG-Fc accessible ICs. Data will be analyzed according to clinical stage of patients, histologic type of tumor, histology of regional lymph nodes, presence or absence of CEA and K-casein, opsonin, and pancreatic oncofetal antigen (POA), B-human chorionic gonadotropin (B-hCG) and prolactin.

Progress: Systems have been developed and are now operational for collection, coding, and storage of blood samples; assembling pertinent clinical data; and for data analysis. IC assays have been perfected and standardized, including modification of the Raji cell assay for use with conventional rabbit anti-IgG antibody to detect cell-bound ICs. Preliminary measurements have been made on sera from approximately 350 patients. Epidemiological analyses of the various patient populations have begun. These include analysis according to age, race, age at menarche and menopause, where applicable; pregnancy data and risk factors. Risk factors include family history of cancers, duration of nursing, years of birth control pills, use of post-menopausal estrogens and exposure to smoking and carcinogens. Correlative analyses between IC levels and clinical data and other laboratory parameters have begun. Changes in IC levels in sera obtained longitudinally from individual patients are being correlated with clinical course.

Project Officer: D. Jane Taylor, Ph.D., Donald E. Henson, M.D.
Program: Breast Cancer Diagnosis
FY 80 Funds: $66,750

CONTRACT RESEARCH SUMMARY

Title: Mass Screening for Breast Cancer by Electronic Infrared Pattern Recognition

Principal Investigator: JoAnn D. Haberman, M.D.
Name/Address University of Oklahoma
Performing Organization: Oklahoma City, Oklahoma 73104

Contract Number: NO1-CB-43869
Starting Date: April 1, 1974 Expiration Date: September 29, 1979

Goal: To design and clinically test a prototype system for mass screening for breast cancer utilizing quantitative thermography and computer processing with automated methods of evaluation.

Approach: This project is Phase II of a proposed three phase development program. The first phase included the following: (1) Construction of an improved thermographic instrument designed with the unique serial scan multiple element detector developed by Honeywell. This instrument provides radiometrically calibrated infrared imagery which is subsequently digitized. The instrument is equipped for direct recording on a standard video recorder and for conventional television display. (2) Initiation of a program of clinical evaluation to assess the utility of various quantitative parameters in selecting the thermographic changes of breast cancer. (3) A time-series evaluation of a group of subjects to obtain estimates of the influence of several physiological factors on the thermal patterns of the breast. Phase II consists of further analysis employing methods of computer analysis (allocation rules) capable of selecting women with cancer from those with normal breasts.

Progress: Initial months of Phase II were devoted to system improvement with incorporation of a 6 bit A/D conversion video digitizer providing "on-line" digitization of the thermal image. Presently, all thermograms are digitized "on-line." To date, 10,084 examinations including 105 cancers are included in the data base. Thermal variables (absolute temperature measurement parameters) based on earlier observations of the composition of qualitative images, breast anatomy, breast physiology and breast heat transfer analysis have been identified. These variables (identifying features) are being examined as diagnostic indicators. A number of allocation rules (discriminating equations utilized in computer processing and automated methods of evaluation) based on a set of 400 normals, 250 benign (biopsied) cases and 105 cancers have been developed. The task of quality review of collected data, tape reduction and processing is nearing completion. Collection of additional cancers is progressing parallel with analysis of data to establish which allocation rules will most effectively discriminate cancer subjects from the normal population.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Working Group
FY 79 Funds: -0- (Terminal)

CONTRACT RESEARCH SUMMARY

Title: Risk Associated with In Situ Carcinoma and "Precancerous" Mammary Hyperplasias

Principal Investigator: Dr. David L. Page
Performing Organization: Vanderbilt University
City and State: Nashville, TN

Contract Number: NO1-CB-74098
Starting Date: 7/15/77 Expiration Date: 4/14/81

Goal: To compare subgroups of females regarding their incidence of breast carcinoma during a 10 to 25 year interval after biopsy diagnosis of in situ carcinoma and mammary epithelial hyperplasias.

Approach: This will be a retrospective cohort study in which slides will be reviewed and classified on over 9,000 breast biopsies performed during 1952-1968. This study population will then be contacted and follow-up information on each subject obtained regarding subsequent development of mammary carcinoma, as well as other pertinent clinical and epidemiological data. The control group will be composed of patients with biopsy but no evidence of proliferative lesions. An effort will be made to identify those specific breast lesions or combinations of lesions which are associated with an increased risk of development of breast cancer. Non-anatomic risk factors will also be correlated.

Progress: There have been 2980 contact letters mailed with 2190 completed questionnaires on file. Operating surgeons and their office personnel have given invaluable aid in these efforts. A data quality assurance program has been designed and implemented. Two programs for statistical analysis have been written and are in place for final analysis. Histologic classification is complete for all biopsies in the study. The total number of biopsies with hyperplastic lesions or carcinoma in situ is 2735. One hundred sixty-six (166) biopsies are identified with carcinoma in situ. One hundred sixty (160) biopsies with atypical lobular hyperplasia and 690 biopsies with severe hyperplasia of ductal type are identified. There are 1,418 biopsies demonstrating ductal hyperplasia of moderate degree.
We have completed review and follow-up of 27 patients with ductal carcinoma in situ treated by diagnostic biopsy only. Three of these patients were followed less than three years, dying of causes unrelated to breast disease or treated by bilateral mastectomy. Of the remaining 24 patients treated with biopsy only, 25% developed invasive breast carcinoma with an average follow-up of 16 years. All invasive breast carcinomas appeared in the same breast demonstrating in situ carcinoma in the biopsy. Five of these were in the same quadrant and one was in an adjacent quadrant. The average interval to invasive carcinoma development was 5.5 years (range 3 to 11 years). The average age at biopsy for all women was 53. Three of the six women developing invasive carcinoma have developed distant metastases, with two of them dying of metastatic breast cancer.

Project Officer: D. Jane Taylor, Ph.D.
Program: Breast Cancer Diagnosis
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Biologic Markers in Breast Cancer: Patient Resource

Principal Investigator:	Dr. Leslie W. Whitney
Performing Organization:	Wilmington Medical Center
City and State:	Wilmington, DE

Contract Number: NO1-CB-74137
Starting Date: 9/1/77 Expiration Date: 8/31/81

Goal: To develop a Breast Cancer Task Force specimen resource for blood from breast cancer patients and controls to be used in a search for and verification of new breast cancer markers.

Approach: Thirty (30) cc's of blood will be collected from all project participants with malignant breast lesions and from those with benign lesions who develop malignancy during the first year follow-up. These specimens will be processed, shipped and stored at -70°C at an NCI-designated blood bank facility with appropriate clinical data.

Progress: In our first contract year (9/1/77 - 8/31/78) 65 patients were entered into the study, 30 of whom have malignant disease. Of these 30, 3 have moved, 2 have refused follow-up and 2 have expired. The remaining 23 all have had a 3rd specimen (2nd annual) drawn and 5 of these 23 have had a 4th specimen (3rd annual) processed.

In our second contract year (9/1/78 - 8/31/79) 247 patients were entered, of which 77 were malignant. Of these 77, 3 have refused follow-up and 5 have expired. A 3rd specimen (2nd annual) of blood has been processed on 41 patients.

As of March 30, 138 follow-up forms have been mailed on a monthly basis to those patients with benign lesions. We have made contact with 123 patients by either mail or telephone.

The total number of vials shipped to Mayo through March 1980 is 4,915.

Project Officer: Ihor J. Masnyk, Ph.D., Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $6,091

CONTRACT RESEARCH SUMMARY

Title: Estrogen Replacement after Premenopausal Oophorectomy and Breast Cancer Risk

Principal Investigators: Dr. Dennis Slone, Dr. Samuel Shapiro
Performing Organization: Boston University Medical Center
City and State: Cambridge, MA

Contract Number: NO1-CB-74099
Starting Date: 9/1/77 Expiration Date: 8/31/81

Goal: To examine the hypothesis that exposure to female hormones, particularly estrogen replacement therapy, is related to an increased risk of breast cancer, either during use or after a latent interval.

Approach: An epidemiological study of the case-control type is being implemented as part of an ongoing, hospital-based, multicenter data collection system. Cases of breast cancer and two control groups are interviewed by trained nurse-monitors in hospitals throughout the country. One control group consists of women with cancers not suspected to be related to female endocrine physiology. The second group of controls comprises hospitalized women with a variety of benign conditions. Information on lifetime drug use and relevant medical data are recorded on standard forms and transferred to computer files for analysis.

Progress: As of the end of March 1980, 1278 women with breast cancer had been studied. Of these 880 (69%) were newly diagnosed, of whom 8% were menopausal because of bilateral oophorectomy. In the analysis, the data will be stratified on risk factors for breast cancer, such as benign breast disease, parity and age at first pregnancy. Women with breast cancer are currently being interviewed at a rate of 600 per year.

In our data*, rates of conjugated estrogen use among potential controls are 3% among premenopausal women, 15% among naturally postmenopausal women, 20% among women menopausal because of hysterectomy who retained at least one ovary, and 39% among women menopausal because of bilateral oophorectomy.

*Rosenberg, L., Shapiro, S., Kaufman, D.W., Slone, D., Miettinen, O.S., and Stolley, P.D.: Patterns and determinants of conjugated estrogen use. Am J Epidemiol 109:676, 1979.

Project Officers: Elizabeth P. Anderson, Ph.D.; Robert Hoover, M.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: $40,000

CONTRACT RESEARCH SUMMARY

Title: Role of Dietary Factors and Non-Contraceptive Estrogens in Breast Cancer

Principal Investigator: Dr. Abraham M.Y. Nomura
Performing Organization: Cancer Center of Hawaii
University of Hawaii
City and State: Honolulu, HI

Contract Number: NO1-CB-53884
Starting Date: 6/30/75 Expiration Date: 12/31/80

Goal: To determine in post-menopausal women if there is an association of breast cancer with specific dietary factors and/or the use of menopausal estrogens.

Approach: A case-control approach is being utilized with a personal interview to collect information on past history of drug usage (including menopausal estrogens) and usual weekly dietary intake. Breast cancer cases from three selected populations (ages 45-74) are identified: (1) the Caucasians in Hawaii, who are at high risk for breast cancer; (2) the Japanese in Hawaii, who are at intermediate risk; and (3) the Japanese in Fukuoka, Japan, representing a low-risk population. For each case, a neighborhood and hospital control, matched by race and age, is interviewed. Attempts are made to verify the drug history with the subject's personal physicians, and a subsample of the study population receives a repeated dietary interview to assess its reliability.

Progress: The study originally aimed to interview 200 cases and 400 controls in each of the three ethnic groups. The Fukuoka portion of the study has been completed with the recruitment of 213 cases, and their respective hospital and neighborhood controls. The percent of refusals were 1%, 4%, and 13% for cases, hospital controls, and neighborhood controls, respectively. In Hawaii, interviews have been completed on 170 cases, 159 hospital controls, and 147 neighborhood controls among the Japanese and 149 cases, 137 hospital controls, and 120 neighborhood controls among the Caucasians, as of March 31, 1980. The current percents of refusals are as follows: 15%, 19%, and 15% for the Japanese and 17%, 20%, and 15% for the Caucasian cases, hospital controls, and neighborhood controls, respectively.

Project Officers: Elizabeth P. Anderson, Ph.D.; David Levin, M.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Correlations Between Dietary Factors and Pre- and Postmenopausal Breast Cancer

Principal Investigator: Dr. Baruch Modan
Performing Organization: Chaim Sheba Medical Center and Tel Aviv University Medical School
City and Country: Tel Hashomer, Israel

Contract Number: NO1-CB-53883
Starting Date: 6/30/75
Expiration Date: 6/30/80

Goal: To carry out epidemiological investigations of selected population groups to elucidate the possible role of diet and/or nutritional status on the incidence, pathogenesis, and natural history of pre- and postmenopausal breast cancer.

Approach: A case-control study using a validated questionnaire is being conducted to investigate the following possible risk factors of pre- and postmenopausal breast cancer: dietary components; weight and height; radiotherapy treatment, breast feeding; long-time drug use; personal and family history of breast disease. The study population is composed of 720 newly diagnosed breast cancer cases (pre- and postmenopausal) of which 450 will be European and American born, 200 will be Asian-African born, and 70 will be Israeli born. There are two control groups: one drawn from hospitals, one from neighborhoods. These are matched to the cases for age, country of origin, and period of immigration to Israel. All analyses will be performed separately for ethnic groups, histopathologic category, and by menopausal status.

Progress: As of October 1, 1979, a total of 2,404 (818 cases, 770 surgical controls, 816 neighborhood controls) interviews have been completed. In addition, 690 (270 cases, 253 surgical controls, 167 neighborhood controls) European-born women above age 50 were interviewed using a shorter version of the questionnaire. This version includes all of the regular questions except the food items; 130 women were re-interviewed to examine reliability of the collected data. Pathology slide review and verification of surgical history is also complete. The computer file of the study population is finished and preliminary analysis on fats, fiber and oral contraceptive use on a sample of 200 triples has been accomplished.

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Benign Breast Disease

Principal Investigator: Dr. Baruch Modan
Performing Organization: Chaim Sheba Medical Center
City and Country: Tel Hashomer, Israel

Contract Number: NO1-CB-74102
Starting Date: 8/1/77 Expiration Date: 7/31/81

Goal: To determine the characteristics of patients with different types of noninvasive breast lesions, with particular reference to diet and to those factors reputed to be preferentially associated with breast cancer, and to compare the findings with our similarly conducted breast cancer study.

Approach: The study consists of two phases: (a) case-control interview study of 600 cases of non-invasive breast diseases in terms of such risk factors as: dietary habits, age at first birth, menstrual history, menopausal status, parity, height and weight, familial breast cancer, selected drug history, and previous radiation exposure; and (b) an incidence study of non-invasive breast lesions in a total community. The characterization of the breast lesions will take account of the histological pattern, degree of atypia, and lymphoreticuloendothelial response (LRE). Particular attention will be paid to the relative frequency of distinct histo-pathological forms in high and low risk sub-populations (i.e. Europeans, Asian-African born).

Progress: As of October 1, 1979, 1,108 interviews have been conducted: 518 cases, 353 surgical controls, and 237 neighborhood controls. All of the interviews have been performed at home. All histopathological slides have been reviewed by at least one of the study pathologists. The frequency distribution of cases by conventional pathological criteria are as follows: fibroadenoma 98, proliferative 200, microcystic 78, combined 10, granuloma 29, other 100, and according to Black's grading system: grade 1 -- 152, grade 2 -- 151, grade 2-3 -- 58, grade 3 -- 36. The pathology review shows that grade 3 is rarer than originally expected. Approximately one-half of the questionnaires have been coded and previous surgery has been validated. An incidence study of the whole spectrum of breast disease was started in July 1979. This study includes demographic and pathological data. All pathology slides are being reviewed using the Black classification. So far 720 cases have been accrued.

Project Officers: Elizabeth P. Anderson, Ph.D.; William Blot, Ph.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: $48,763

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Pre- and Postmenopausal Breast Cancer

Principal Investigator: Dr. David C. Deubner
Performing Organization: Duke University Medical Center
City and State: Durham, NC

Contract Number: NO1-CB-63996
Starting Date: 6/30/76 Expiration Date: 2/28/81

Goal: To define the epidemiologic characteristics of breast cancer in pre- and postmenopausal women, and of breast cancers rich and poor in cytoplasmic receptors for estrogen and progesterone.

Approach: This case-referent study is of breast cancer patients undergoing surgery at university and community hospitals, surgical and medical patients not having breast or gynecological surgery at the same hospitals, and subjects from the community. Data collection is by interview of all subjects and review of charts of hospital patients. Laboratory studies on tissue and serum from breast cancer patients include quantitative estrogen and progesterone receptor determinations, evaluation of histology and ultrastructure by a standardized protocol, and estrogen and progesterone serum determinations. Statistical analyses estimate the relative strength of association between breast cancer in pre- and postmenopausal women and such factors as age of menarche, age of first delivery, obesity, and family history of breast cancer. Associations are also being sought between these factors and concentrations of the cytoplasmic hormone receptors.

Progress: Collection and computer entry of interview, hospital chart review, and estrogen receptor data is complete. Light microscopy review is near completion and ultrastructure review is well underway. Analyses of interview data relevant to the epidemiology of pre- and postmenopausal breast cancer are complete and are being prepared for publication. Preliminary analyses of the epidemiology of estrogen receptor content of tumors are in progress.

Project Officers: Elizabeth P. Anderson, Ph.D.; Gordon B. Cutler, M.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: $40,000

CONTRACT RESEARCH SUMMARY

Title: Endocrine Events at the Time of First Pregnancy

Principal Investigator: Dr. John R.K. Preedy
Performing Organization: Emory University School of Medicine
City and State: Atlanta, GA

Contract Number: NO1-CB-74104
Starting Date: 8/1/77 Expiration Date: 7/31/81

Goal: It is proposed that the protective effect of an early first pregnancy against breast cancer is due to a change in the hormonal environment following that event. We proposed to investigate and characterize such a change.

Approach: Nulliparous subjects in the 18-22 age range who have not taken birth control pills, who have a history of normal regular menses, and who are planning pregnancy in the next 1-2 years will be selected (Group A). Similar subjects will be chosen who do not plan to become pregnant in the next 1-2 years (Group B). Similar subjects to the above but in the age range 30-40 would also be selected (Groups C and D). All groups would have the following carried out in the early follicular phase of the menstrual cycle: plasma gonadotrophins, estrogens, progesterone, androgens; urine estrogens and androgens; plasma protein-steroid binding studies; perphenazine-prolactin stimulation test; LRH-LH stimulation test. Results from Groups A, B, C and D would be compared by appropriate analysis of variance techniques to determine significant contrasts among the four groups.

Progress: We have finished with recruitment of subjects as stated in the last report. As of 5/14/80, the total number of subjects participating in the program are: Group A - 41 (initial study has been done on all 41. 19 have become pregnant and 4 of these have suffered miscarriage. 7 have reached full term and delivered and the remaining 8 are still pregnant. None of the subjects who have delivered has been restudied yet.) Group B - 29 (one other subject in this group decided not to participate. All 29 have undergone the initial study and 2 have been restudied). Group C - 24 (one other subject initially in this group decided not to participate. All 24 have undergone the initial study. 9 have become pregnant and one of these has suffered a miscarriage. 3 have so far delivered and none of these has been restudied). Group D - 28 (2 others initially in this group decided not to participate. All 28 have undergone the initial study and 4 of these have been restudied).

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: $135,000

CONTRACT RESEARCH SUMMARY

Title: Pathogenetic Models of Malignant and Premalignant Disease of the Breast

Principal Investigators:	Dr. John A. H. Lee Dr. Suresh H. Moolgavkar
Performing Organization	Fred Hutchinson Cancer Res. Center
City and State	Seattle, WA

Contract Number: NO1-CB-74100
Starting Date: 9/1/77 Expiration Date: 8/31/80

Goal: To translate hypotheses about the development of breast cancer into mathematical terms, and to test the predictions of these against data on the epidemiology of the disease.

Approach: The behavior of the incidence curves generated by models will be studied under perturbations of the parameters. Maximum likelihood estimations will be used to fit the model to population data which have been statistically analyzed to allow for the effects of secular trends on age distribution.

Progress: We have proposed a biologically based, two-stage, mathematical model for carcinogenesis which relates events occurring at the cellular level to epidemiologic features of breast cancer in females. This model, which accomodates the physiological responses of the breast tissue to menarche, menopause, and pregnancy, predicts age-specific incidence curves that are in close quantitative agreement with those observed in high, intermediate, and low risk populations. The model predicts a protective effect of early first birth that is in good quantitative agreement with data from a multinational study. The epidemiology of breast cancer is consistent with the view that pre- and post-menopausal breast cancers are identical from the point of view of pathogenesis. A paper describing this model is to appear in the September, 1980, issue of JNCI. Under this contract, we have published papers showing that (1) the age-specific incidence and mortality for breast cancer in males resemble those for common, epithelial tumors in U.S. whites (high risk) and in Japanese (low risk), (2) the Armitage-Doll approximation to the multistage theory of carcinogenesis can be improved, and (3) a two-stage model for carcinogenesis can describe the qualitative features of the whole spectrum of human cancer incidence curves. Finally, we described methodology for studying temporal trends in cancer and showed that, after adjustment for these trends, breast cancer incidence curves have similar shapes in high and low risk populations. This is in striking contrast to the observations from cross-sectional (unadjusted for temporal trends) data.

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Lipid and Cholesterol Levels in Relation to Human Breast Cancer Risk

Principal Investigator: Dr. Gary D. Friedman
Performing Organization: Kaiser Foundation Research Institute
City and State: Oakland, CA

Contract Number: NO1-CB-84238
Starting Date: 8/7/78 Expiration Date: 2/6/80

Goal: To explore a possible etiologic role of dietary fat in breast cancer by examination of breast cancer incidence in relation to previous levels of serum cholesterol and other serum lipids.

Approach: We compared incidence rates of breast cancer and associated cancers in groups of women defined by their serum cholesterol and other serum lipids at a time prior to their diagnosis. Using data from the Northern California Kaiser Foundation Health Plan's multiphasic health checkups (over 95,000 women between 1964 and 1972), we estimated that more than 250 cases of breast cancer would be available for review. Medical data on cholesterol levels, β-lipoprotein, and total lipids were available. Known and available risk factors and possible confounding variables, such as obesity and ethnic background, were considered in analysis.

Progress: This study was completed in February, 1980. The age-adjusted rate of breast cancer in a cohort of 95,179 women was 1.38 per 1000 person-years. For comparison, the rate for breast cancer in women over 15 years of age in the Third National Cancer Survey in the San Francisco-Oakland Standard metropolitan Statistical Area was also 1.38 per 1000 women age adjusted to the same standard. Total follow-up was 689,602 person-years. Incidence rates of breast cancer were 1.41, 1.43, 1.31, and 1.37 per 1000 person-years from the lowest to the highest quartile of serum cholesterol level, respectively. Likewise, no relationship was detected between β-lipoprotein or total lipids and breast cancer. The validity of this lack of association was strengthened by the presence of the expected relationship of breast cancer to established risk factors such as age at menarche and menopause, parity and educational level. Multiple logistic analysis, which included ten covariables, failed to uncover any hidden relationship between serum cholesterol and breast cancer. These data suggest that the hypothesized causal relationship between dietary fat and breast cancer does not act via an effect on circulating lipid levels.

Project Officers: Elizabeth P. Anderson, Ph.D.; Helen Hubert, Ph.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: The Relationship Between Thyroid Disease and Breast Cancer

Principal Investigator: Dr. Farahe Maloof
Performing Organization: Massachusetts General Hospital
City and State: Boston, MA

Contract Number: NO1-CB-84230
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: To examine the possible association of breast cancer with prior thyroid dysfunction.

Approach: This is a retrospective cohort study designed to measure the rates of breast cancer in women with and without prior thyroid disease. A mortality study will follow 7,000 women with thyroid disease seen at the Thyroid Clinic of the Massachusetts General Hospital between 1925 and 1975, and 7,000 matched population controls; 2,000 women seen at the Clinic but without identified thyroid disease will also be followed. Rates will be compared in women with various types of thyroid disease. A morbidity study will follow 1,600 women with previous thyrotoxicosis; this will include 500 women who became hypothyroid following the treatment and 500 who did not. Rates of breast disease will be compared according to mode of treatment as well as externally compared with rates from the Connecticut Tumor Registry.

Progress: Mortality Study: The card file of patients treated at the Thyroid Clinic was reviewed. From the 45,000 names, 13,400 women satisfied the study criteria, almost twice as many women as had been anticipated, allowing us to increase the sample size. This included 3,140 women with no thyroid disease. The information was coded, keypunched, and entered into the computer. A form to abstract the medical records has been developed. To date 4,000 medical records have been abstracted. In addition, a follow-up form to record information useful to trace the patient has been drawn up. This form has been completed for 4,000 women. One control woman was sought from the available Massachusetts Residents' Lists for each thyroid patient who resided in the state at the time of diagnosis; 10,200 women who resided in the same neighborhood and who were within two years of age of the case were selected. The information present in the Residents' Lists was abstracted, keypunched, and entered into the computer for each control women selected. A search has begun of Massachusetts death certificates for the cause of death of any deceased thyroid patients or controls.

Morbidity Study: A computerized roster of the 1,955 women in the USPHS Thyrotoxicosis Follow-up Study has been compiled. A search of the Massachusetts death certificates found 389 deaths. A questionnaire was mailed to 1,566 women on April 2, 1979. A second mailing to those who did not respond went out May 28, 1979. To date, 75% of the 1,955 women have been found dead or have returned a completed questionnaire. Those who have not yet responded are being traced through the medical records, telephone books, and Residents' Lists. The scheduling and supervision of the clinical examinations are well underway. So far, 305 patients have been examined. The USPHS Thyrotoxicosis Follow-Up Study records are being coded. Thus far, 75 records have been abstracted.

Project Officers: Elizabeth P. Anderson, Ph.D.; Bruce Nisula, M.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: $171,800

CONTRACT RESEARCH SUMMARY

Title: Lipid Levels and Cholesterol Metabolism in Relation to Human Breast Cancer Risk

Principal Investigator: Dr. Angelos E. Papatestas
Performing Organization: Mount Sinai Medical Center
City and State: New York, NY

Contract Number: NO1-CB-84318
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: To evaluate whether environmental factors, such as diet, influence serum lipid levels and metabolism and produce systemic or local alterations which may be causally related to breast cancer risk.

Approach: A pair-wise matched case-control study. Matching criteria: age, race and menopausal status. Data on past medical and family history, dietary habits, lipid levels and cholesterol metabolism in patients with breast cancer and in controls (patients and volunteers) are analyzed. Correlations between these parameters and epidemiological risk factors, or tumor characteristics are examined. Data on tissue enzymes, fat cell size and hormone receptors are examined for correlations with lipid levels.

Progress: Significant differences between cases and controls were noted in:

PARAMETERS	# OF PAIRS	CONTROLS	CASES	MEAN DIFFERENCE	T-TEST	P
U.S. WOMEN						
Serum Cholesterol (CHOL,mg/dl)	78	194±42	209±36	-14±52	2.52	<0.02
Serum Triglycerides (TRIG,mg/dl)	67	100±63	124±84	-24±21	2.15	<0.05
Serum Free Fatty Acids (FFA)(mEq/l)	64	.29±.10	.34±.13	-.4±.17	2.14	<0.05
CRI: CHOL+TRIG+Weight / Quetelet's Index (QI)	72	126±127	140±30	-14±37	3.39	<0.01
Lipid Index: CHOL+TRIG+FFA/QI	65	123±49	156±54	-32±61	4.21	<0.001
GREEK WOMEN						
Serum Triglycerides	40	74±36	118±61	-43±69	3.98	<0.001
CRI	40	116±19	128±24	-12±35	2.26	<0.05

In U.S. women differences in cholesterol and in the composite risk index (CRI) were more significant in the postmenopausal group and in ethnic minorities while differences in the lipid index were significant in white women and the premenopausals as well. Differences in cholesterol were more significant between controls without benign breast disease and cases while differences in the CRI were more significant in controls with benign breast disease and cases. In Greek women differences in TRIG were more significant than cholesterol differences. Hyperlipidemia was associated with a significantly increased relative breast cancer risk. Marginal X^2 test: 9:00, $p<0.01$. Differences in stool steroid excretion and in the target organ were less significant than those observed in the serum. The findings suggest involvement of lipids in the disease pathway at the systemic level. The observed variations between subgroups with different dietary habits and environmental influences, that are also different in breast cancer risk, provide further evidence for the impact of environment on the development of breast cancer.

Project Officers: Elizabeth P. Anderson, Ph.D.; Kenneth Lippel, Ph.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: $225,200

CONTRACT RESEARCH SUMMARY

Title: Interrelationships Among Diet, Steroid Hormone Metabolism, and Human Breast Cancer

Principal Investigator: Dr. Marvin A. Kirschner
Performing Organization: Newark Beth Israel Medical Center
City and State: Newark, NJ

Contract Number: NO1-CB-84229
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: To investigate steroid hormone production rates and metabolism in relation to obesity and dietary patterns.

Approach: This will be an in-depth study of androgen, estrogen and cortisol production, metabolism, and excretion in a cohort of 40 obese women undergoing drastic weight reduction and diet manipulation. The women will be studied prior to weight loss, after weight loss and stabilization on a high protein, low fat carbohydrate diet, and after isocaloric exchange to a more typical high carbohydrate, high fat diet. Values will be compared under three sets of conditions and further compared with those in lean, age-matched controls.

Progress: Baseline studies have been performed on 46 obese and 28 normal women. Approximately, half of these studies have been worked up to date, as follows:

	Obese	Normal Controls	p Value
MCR Androstenedione (Δ)	2870 L/day	2140	<.001
Plasma Δ	108 ng/dl	116	N.S.
Δ Production Rate	3.03 mg/day	2.45	<0.10
Conversion $\Delta \rightarrow E_1$	2.69%	1.54%	<0.05
Conversion Δ→Testosterone	7.5%	12.4%	<0.05
Urinary E, Prod. Rate	201.5 µg/day	101.6	<0.10

These data demonstrate increased clearance and production rates of Δ, altered metabolism of Δ to both testosterone and E_1, and increased urinary E_1 production. Some of the values are not yet significant due to large variance in the obese group, but may become significant when more data points are available.
Peripheral conversion of Δ^4-androstenedione to androsterone and etiocholanolone and excretion of the metabolites seem no different in obese vs. normal women.
To date 17 normal volunteers have been studied during normal, high protein, and high carbohydrate diets. Although dietary analyses confirm patient compliance, we cannot see any significant differences in Δ^4 production, peripheral metabolism and excretion, or E_1 production on these differing diets.
Our major problem encountered to date is the very high dropout rate in the obese women prior to stabilizing at ideal body weight. Although most patients have lost considerable weight, (m = 44 lbs), only 3 patients to date have reduced to ideal body weight and have been stabilized sufficiently to enable the second and third studies to be performed. This aspect of the study may require a longer period of time to complete data acquisition.

Project Officers: Elizabeth P. Anderson, Ph.D.; Lynn Loriaux, M.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: $131,400

CONTRACT RESEARCH SUMMARY

Title: Relation of Intestinal Flora to Breast Cancer in Humans

Principal Investigator: Dr. Sherwood L. Gorbach
Performing Organization: New England Medical Center
City and State: Boston, MA

Contract Number: NO1-CB-74104
Starting Date: 7/1/77 Expiration Date: 12/31/80

Goal: To determine the influence of diet and other risk factors on the metabolism of estrogens by bacteria in the large bowel.

Approach: The estrogen profiles are determined in the serum, urine and feces of young and older women eating "Western" or vegetarian diets and in women with a history of breast cancer. Novel fecal metabolic transformation of estrogens and the possible role of these transformations in the etiology of breast cancer is being investigated

Progress: The enrollment of subjects has been completed; four 72-hour collections of urine, feces, serum, and dietary intake over a one-year period, have been obtained for all subjects. The main effects being analyzed are ovarian status (young, menstruating women versus post-menopausal women) and diet (vegetarian versus omnivores). A fifth group of post-menopausal subjects with breast cancer is also being studied. All three-day dietary records have been coded and are ready for computer processing. Cholesterol and fatty acid values for all foods in the food dictionary have been added. Baseline data (e.g., dry weights, protein content, etc.) on the 72-hour urine and feces samples have been completed. Approximately two-thirds of the 72-hour urine, feces and serum samples have been sent to Finland, with all but a last batch processed to determine estrogen content. These results indicate a variety of interesting group differences about which we are confident, but in some instances we do not have a precise estimate of their magnitude as yet. The fecal extraction from vegetarians is twice as much as from omnivores (in terms of dry weight), and the older women excreted about 70% as much as the young women. Vegetarians excrete three times as much unconjugated E_1,E_2 and E_3 in their feces as the omnivorous women, regardless of diet. A striking feature of these results is the remarkable similarity among the three estrogens. The vegetarians excrete 81% as much estriol-16-glucuronide as the omnivores, and as expected, the post-menopausal women excrete 38% as much estriol-16-glucuronide as the young women. The preliminary results also indicate that the rate of in vitro fecal hydrolysis of estradiol-3-glucuronide is 15-20% higher in omnivores (fecal bacterial β-glucuronidase activity). The results suggest that vegetarians reabsorb biliary estrogens to a lesser extent than omnivores, thus making these estrogens more available for intestinal bacterial transformation and fecal elimination.

Project Officers: Elizabeth P. Anderson, Ph.D.; Carl Smith, Ph.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: $21,800

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Minimal Breast Cancer in Initially Asymptomatic Women

Principal Investigator: Dr. Bernard S. Pasternack
Performing Organization: New York University Medical Center
City and State: New York, NY

Contract Number: NO1-CB-74103
Starting Date: 7/15/77 Expiration Date: 12/31/80

Goal: Investigate by means of risk factor analysis whether minimal breast cancer in initially asymptomatic women is essentially different from clinical breast cancer or whether it is an early manifestation of the same disease; identify risk factors associated with tumor aggressiveness.

Approach: We are obtaining and will analyze demographic and medical data recorded at the Guttman Breast Diagnostic Institute, which since 1968 has been offering free breast examinations to women living in New York City. The study group will consist of an estimated 1054 valid cases out of a total of 1403 detected through November 21, 1979 and approximately 2248 valid controls out of a random selection of 2700. After exclusions due to symptomaticity, 587 cases and 1704 controls will remain. Two independent histopathologic diagnoses are being performed; one at New York University Medical Center (NYUMC) and the other at Saint Barnabas Medical Center (SBMC). Cases will be compared according to three tumor criteria: a) level of invasiveness, b) level of aggressiveness and c) histologic type. Comparison of all cases to controls should result in estimates of relative risk within the range found at other screening centers, whereas the comparison of clinical cases to controls should provide estimates within the same order of magnitude as registry data.

Progress: Protocols have been established for all phases of the study: collection of demographic and screening data, pathology review, and mammography review. Basic identification forms have been coded, keypunched, and filed for all 1403 cases, and coded for 2133 controls. Examination code forms for about 3640 participants have been coded; of these, 3240 keypunched; and of these 2700 filed. Initial and recall histories have been coded for about 3280 participants; of these, 2600 keypunched; and of these, 2600 filed. Identification of inconsistencies in the data by means of a specifically designed computer program has been performed for 5480 examination and history forms. These inconsistencies are being resolved by examination of participants' files at both NYUMC and the Guttman Institute. Pathology material has been requested for 687 cases and received for 483 (70%). Mammography review has been performed for 307 cases and 42 controls, the NYUMC pathology review for 368 cases, and the SBMC pathology review as well for 336 of these cases. Of those for which two reviews have been completed, the initial rate of consensus is 31%. Consensus review has been able so far to resolve all remaining differences.

Project Officers: Elizabeth P. Anderson, Ph.D.; Benjamin Hankey, Sc.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Medullary and Lobular Breast Cancer

Principal Investigator	Dr. Paul P. Rosen
Performing Organization	Sloan-Kettering Institute for Cancer Research
City and State	New York, NY

Contract Number: NO1-CB-63997
Starting Date: 6/30/76 Expiration Date: 12/29/80

Goal: To identify and describe morphologic features of human female mammary carcinoma which are significantly related to known risk factors for the development of such carcinomas.

Approach: Detailed epidemiologic information will be obtained by interview from women with mammary carcinoma and correlated with pathologic features of the tumors. The analysis of the data will emphasize comparison of medullary and lobular carcinoma with duct carcinoma but will also permit the study of morphologic features not limited to one tumor type.

Progress: A total of 1,227 patients have been accessioned into the study. Among the cases reviewed, 81 women were found to have only medullary carcinoma, 38 only in situ lobular carcinoma, and 59 only infiltrating lobular carcinoma. In addition, 42 patients had lobular carcinoma (38 invasive, 4 noninvasive) combined with another type of carcinoma, and 11 patients had medullary carcinoma associated with another type of carcinoma. Interviews have been conducted with 98% of the patients. Data verification has been accomplished for 75% of prior medical and surgical treatments and additional information continues to be received. All currently available data on each patient have been entered into the computer. Analysis of correlations between morphologic and epidemiologic observations is in progress.
We have observed that patients with medullary carcinoma had a significantly lower mean age at diagnosis than those with duct and lobular carcinoma. There was a disproportionately high number of non-whites in the medullary carcinoma group. A secondary correlation with medullary carcinoma was a higher frequency of birth in the southern part of the United States as well as in South and Central America. The number of Oriental women was too small for analysis. Patients with medullary carcinoma tended to weigh more at diagnosis than those with other types of carcinoma, but the differences were not significant when evaluated in terms of age and optimum weight. No correlation between height or height-weight indices and tumor type was observed.
Preliminary analysis of methods of clinical examination indicates significant relationships between the frequency of physician and mammographic examination and tumor type. Thus far, no correlation between self-examination and tumor histology has been found.

Project Officers: Elizabeth P. Anderson, Ph.D.; Roger Connelly
Program: Breast Cancer Epidemiology
FY 80 Funds: $18,069

CONTRACT RESEARCH SUMMARY

Title: Correlations Between Morphology and Epidemiology of Breast Cancer

Principal Investigator: Dr. Björn Stenkvist
Performing Organization: University Hospital, Uppsala
City and Country: S-750 14 Uppsala, Sweden

Contract Number: NO1-CB-53968
Starting Date: 6/30/75 Expiration Date: 6/30/81

Goal: To ascertain possible correlations between morphologic variables such as cytologic and histopathologic classifications and established epidemiological risk factors of breast cancer.

Approach: Within a clearly defined area and population (4 Swedish counties) 181 breast cancer cases occurred during a defined study period (5 months). From 179 of these patients both epidemiological and morphological data could be obtained. From 179 exactly age-matched controls epidemiological data were recorded in the same way. The tumors were characterized by computerized cytometry of the cell population in the tumors as well as by conventional morphology, resulting in an objective atypia measure.

Progress: Comparison between patients and controls showed no significant differences in age at menarche, age at first birth, age at menopause, number of children, height, weight, obesity, or frequency of diabetes or hypertension. These results differ from most earlier reports, possibly because the controls here were selected from the whole female population instead of hospitalized patients. Heredity and frequency of prior breast disease were significantly higher in the patient group. Data on thyroid hormone levels suggested that altered thyroid function in the patients was probably due to a change in the peripheral conversion of thyroxin. The patients had higher serum concentrations of estrone, androstenedione, and testosterone, and a lower level of sex-hormone-binding globulin. No obvious differences in the epidemiological characteristics could be discerned among different conventional morphological subgroups. The computerized cytometry value was positively correlated with biological parameters such as mitotic rate and had significantly higher reproducibility than subjective systems for classification or grading. The patients have been followed up for the first thousand days post-mastectomy. A "malignancy grade" for breast carcinomas has been developed based on image analysis and pattern recognition. Patients with tumors over the mean of this value had 39% recurrences within the first thousand days of observation; patients with tumors below this mean value 0%. This "high-risk" index for the first thousand days of follow-up was superior to other risk indices such as level of carcino-embryonic antigen, estrogen and progesterone receptor levels, as well as TNM-stage. The study is continuing with comprehensive epidemiological analyses of subgroups and continued analyses of factors influencing survival between 1000 and 2000 days after mastectomy.

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Further Linkage Analysis of Families with High Incidence of Breast Cancer

Principal Investigator: Dr. Mary Claire King
Performing Organization: University of California, SF
City and State: San Francisco, CA

Contract Number: NO1-CB-84274
Starting Date: 6/1/78 Expiration Date: 11/30/79

Goal: To confirm the existence of postulated alleles influencing cancer susceptibility in certain families with very high breast cancer incidence.

Approach: In collaboration with Dr. R.C. Elston, University of North Carolina, we are using maximum likelihood methods of linkage analysis to map hypothetical alleles for breast cancer susceptibility with respect to seven newly developed marker loci. The 15 families under investigation, and the genetic models which best explain the distribution of breast cancer in each, have been developed in the course of our collaboration with Dr. H.T. Lynch (contract CB-44003). The present project strengthens that approach by adding markers not available at the time of the original contract.

Progress: During the past year, we have accumulated evidence that a dominant allele greatly increasing susceptibility to breast cancer is linked to the genetic marker GPT, probably on chromosome 10. It is therefore important to determine whether any of the markers analyzed in the present contract appear to be linked to the susceptibility allele in these same families. None of the markers analyzed under this contract are on chromosome 10, so evidence against linkage of these markers to the putative susceptibility allele would increase support for the linkage of this allele to the GPT locus. The lod scores for linkage of five of the seven genetic markers in this analysis to the hypothetical dominant allele increasing susceptibility to breast cancer in families with high incidence of the disease are presented below. Two other markers (protease inhibitor and peptidase A) are still under investigation.

	recombination fraction					
locus	0.0	0.1	0.2	0.3	0.4	0.5
Amylase 2	- .15	-.05	-.02	-.00	-.00	0
Esterase D	-3.12	-.85	-.32	-.09	-.00	0
Glyoxylase I	-2.91	-.67	-.10	+.06	+.04	0
Properdin factor B (Bf=GBG)	-2.12	-.51	-.16	-.06	-.04	0
Transcobalamin II	-1.29	-.50	-.18	-.04	+.00	0

Finally, we have used these data to tentatively map the transcobalamin II locus on chromosome 9, near the genetic marker adenylate kinase. This result was presented at the 1979 Human Gene Mapping meeting. The breast cancer linkage data has been published (Science 208: 406, 1980).

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Pre- and Postmenopausal Breast Cancer

Principal Investigator: Dr. Ralph S. Paffenbarger, Jr.
Performing Organization: University of California, Berkeley
City and State: Berkeley, CA

Contract Number: NO1-CB-63998
Starting Date: 6/30/76 Expiration Date: 6/29/80

Goal: This work identifies host and environmental characteristics that predispose to the development of breast cancer in women of all ages, and contrasts the findings between premenopausal and postmenopausal women.

Approach: Using epidemiological methods of case-control design, we are conducting structured interviews with women in the San Francisco Bay area, totaling over 2,500 breast cancer patients and 5,000 control women. Characteristics to receive special attention as potential precursors of breast cancer include age, catamenial and childbearing events, indicators of early fertility, exogenous hormone and drug use, sociocultural factors, family history of cancer, radiation exposure, and various physical measurements. Extent of disease (staging), histological diagnoses, and survival determinations are being established.

Progress: Interviews have been completed on 5,259 study subjects, 1,868 of whom are breast cancer patients of all ages, and 3,391 of whom are age-, race-, and hospital-matched control patients. Analyses have been made that examine potential characteristics of high risk in pre- and postmenopausal women. Common to increased risk of this disease in both periods of womanhood were early menarche and late menopause; delayed marriage and first childbirth; more nulliparity or reduced gravidity and parity; reduced frequency of abortion; longer intervals between first and second childbirth, and shorter overall childbearing interval; more advanced education, higher socioeconomic status and more contraceptive usage; and familial tendencies toward the disease. Breast cancer patients diagnosed before menopause were leaner than controls at age 20 and at time of diagnosis, but breast cancer risk in the postmenopausal period was related to increased weight-for-height at diagnosis and greater weight gain since age 20. Frequency and duration of the gravid state, inversely related to breast cancer risk, were largely dependent on contraceptive practices rather than unexplained infertility per se.
Pathological classification of ductal atypia (Black-Chabon) in 728 patients with breast cancer and 444 patients with benign breast disease is complete; analyses to date suggest that the decrease in benign breast disease risk among oral contraceptive users is strongest for those with severe atypia, whereas any increase in breast cancer risk among oral contraceptive users would be strongest for those with severe atypia. These findings may depend on age at first childbirth. Analyses of the risk of breast cancer vs. years of reproductive potential are underway and show an increased risk in women with longer total years of ovulation (age held constant). The increase remains when data are examined by age, age at first childbirth, parity, menopausal status, or oral contraceptive usage.

Project Officers: Elizabeth P. Anderson, Ph.D.; Bruce Stadel, M.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Benign and Noninvasive Breast Disease in Populations at Different Risk for Breast Cancer

Principal Investigator: Dr. Nicholas L. Petrakis
Performing Organization: University of California, S.F.
City and State: San Francisco, CA

Contract Number: NO1-CB-84319
Starting Date: 9/30/79 Expiration Date: 7/31/80

Goal: To ascertain the prevalence and spectrum of clinical benign breast disease (BBD) across ethnic groups whose breast cancer rates are known to vary and to explore possible associations with known or suspected breast cancer risk factors. In addition, to investigate cytologic measures which might serve to distinguish women with benign breast disease.

Approach: Clinical breast examination and mammographic findings are being analyzed in relation to sociomedical information collected in a large, regional breast screening program for white, black, Chinese and Japanese women. For a subset of these women, breast fluid specimens obtained through nipple aspiration will be examined cytologically for correlation with clinical breast disease status. We are also examining data collected from nearly 5,000 women since 1972 for our ongoing epidemiologic studies of breast cancer.

Progress: For the screening center population estimates of the frequency of BBD vary widely depending on the measure chosen (i.e., breast nodularity, thickening, or a mass on physical exam, or dysplasia or other anomaly on mammogram). By most measures BBD is more common in whites, but the relative rankings of black and Asian women vary across measures. Mammograms revealed abnormality more than did physical exams. Findings of nodularity decreased as breast size increased but nodularity was not related to dysplasia on mammogram. In postmenopausal women, nulliparity was associated with abnormal mammographic findings but not with palpable nodularity or thickening. Other factors examined include family history of breast cancer, education, age at menarche and pregnancies, lactation history, and breast fluid secretor status.
From data relevant to BBD collected in other ongoing studies, we have examined the frequency of clinically palpable breast conditions--including fibrocystic changes, discrete cysts, and fibroadenomas--by selected epidemiologic characteristics. Our preliminary findings include: a) that fibrocystic changes and discrete cysts were more common among white than black or Asian women; b) that these two conditions peaked among women aged 35-54 and declined in frequency at older ages; c) that within the age range 35-54, fibrocystic changes and discrete cysts were more frequent among pre- than postmenopausal women; d) that among women under the age of 55, those with these two conditions were more likely to be nulliparous than women with no such findings; e) that women with fibrocystic changes or discrete cysts were less likely to be overweight than women with no such findings; and f) that women with fibrocystic changes were more likely to secrete breast fluid than women with no such findings.

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Benign Breast Disease

Principal Investigator:	Dr. Gary H. Spivey
Performing Organization:	University of California,Los Angeles
City and State	Los Angeles, CA

Contract Number: NO1-CB-74202
Starting Date: 8/15/77 Expiration Date: 8/14/81

Goal: To examine the epidemiology of major types of benign breast disease and to compare identifiable risk factors for benign diseases with known risk factors for breast cancer. To examine a histologic classification scheme based on grading of cellular atypia in comparison to the conventional histologic classification.

Approach: Women with first biopsies for benign breast disease in participating hospitals are identified from pathology records and admitted to the study. They are selected to maximize representation of various histologic subtypes. Biopsy slides of participants are reviewed by two pathologists and classified according to two classification systems. A subcategory of women with breast cancer is included. Two types of controls are used, a hospital control and a friend control. Information on epidemiologic characteristics is obtained by a questionnaire covering a wide range of topics including evnironmental influences; familial cancer patterns; medical, maturation, and reproductive history; and other endocrine-related subjects. Epidemiologic characteristics of different histologic types of benign breast disease will be compared to each other and to those of breast cancer.

Progress: Recruitment of study subjects, interviews, and slide review are proceeding at a steady pace near that originally projected. Nevertheless, a request for an extension of the contract has been necessary because of the delay resulting from OMB clearance requirements for the questionnaire. As of May 12, 1980, we have interviewed 710 benign cases, 193 cancer cases, 204 hospital controls, and 271 friend controls. Interviews are currently pending on an additional 109 benign cases, 5 cancer cases, 136 hospital controls, and 86 friend controls who have agreed to participate in the study. We anticipate completion of data collection in November 1980.

Project Officers: Elizabeth P. Anderson, Ph.D.; B. J. Stone, Ph.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: $62,800

CONTRACT RESEARCH SUMMARY

Title: Lipid and Cholesterol Levels and Breast Cancer Risk in Tecumseh Women

Principal Investigator: Dr. Millicent W. Higgins
Performing Organization: University of Michigan
City and State: Ann Arbor, MI

Contract Number: NO1-CB-84317
Starting Date: 9/30/78 Expiration Date: 9/30/80

Goal: The purpose of the research is to identify precursors of breast cancer in women. Cholesterol and triglyceride levels and other known or suspected risk factors will be related to the incidence of breast cancer and the prevalence of benign breast disease in the female population of Tecumseh, Michigan.

Approach: The population includes all women aged 20 or older when examined initially. These 3,000 women are being followed up in studies of chronic obstructive lung disease or breast cancer. All cases of breast cancer will be confirmed by review of medical and hospital records. There are a total of 65 cases of breast cancer. An age-matched control group, three controls per case, has been selected. Additional medical history details on risk factors for breast cancer is being collected for the cases and controls. The entire population of 3,000 women will serve as another comparison group for all items of existing data. The data will be analyzed for possible relationships between cholesterol and triglycerides, and breast cancer incidence, with appropriate evaluation of other etiologic factors as well.

Progress: Review of hospital records and death certificates confirmed breast cancer in 37 living women and 28 dead women; 4 possible cases were found not to have breast cancer. Information about date of diagnosis, histologic type of cancer and treatment was abstracted from hospital charts. Three age-matched controls were selected for each case by a random process and attempts were made to complete 278 medical history forms; relatives of deceased cases and controls were interviewed when possible. Two cancer cases refused to take part in the study and no source of additional information could be found for 6 deceased cases. Forms were completed for 171 controls. The new information pertains to breast cancer, and other breast diseases and their treatment, reproductive history, irradiation, use of female hormones, including birth control pills, and of hypotensive medication, and family histories of breast cancer and of benign breast disease. Information has been key-punched and quality control procedures, and consistency checking will be completed by the end of May. Comparison of dates of diagnosis of breast cancer and dates of examination in the Tecumseh study showed that 52 of the 65 cases were examined before the detection of their cancer; however, the interval between the two events was less than a year in 3 cases. Comparisons can therefore, be made of 49 cases and approximately 3000 other women in prospective analyses of available data designed to identify risk factors for breast cancer. All 65 cases will be included in retrospective analyses of characteristics reported to be present in the past.

Project Officers: Elizabeth P. Anderson, Ph.D.; Helen Hubert, Ph.D.
Program: Breast Cancer Epidemiology
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Benign and Noninvasive Breast Lesions in Groups at Different Breast Cancer Risk

Principal Investigator: Dr. Sue Amelia Bartow
Performing Organization: University of New Mexico
City and State: Albuquerque, NM

Contract Number: NO1-CB-84231
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: To investigate whether population groups with low incidence of breast cancer also have a low prevalence at autopsy of benign breast lesions.

Approach: Combining the resources of the population-based New Mexico Tumor Registry, the State's Medical Investigator's Office and the Medical School Department of Pathology, we propose to examine and compare benign breast lesions in three ethnic groups in an autopsy series--Anglos, Spanish Americans, and American Indians. These three groups are all at differing risk for breast cancer, and the incidence rates and histopathologic distributions of breast cancers are already known from Tumor Registry data. The autopsy series to be examined will include about 500 women in three years, 230 Anglo, 140 Spanish American, and 130 American Indian. Breast lesions will be classified pathologically. Radiological studies of the specimens by both clinical mammography and high resolution techniques will be compared with histopathological findings. Medical, reproductive, and related history of each case will be obtained from the family physician or medical records if at all possible.

Progress: One hundred and fifty-two cases have been accessioned into the study (91 Anglo, 39 Spanish Am., 22 Indian). The ethnic breakdown is thus far skewed in favor of the Anglo. We are, therefore, beginning to selectively perform autopsies more frequently on Spanish and Indian women who fall under the jurisdiction of the State Office of the Medical Investigator. Medical histories on the first 130 cases include 55 completed interviews, 33 unable to complete and 43 pending.
Pathologic and radiologic analysis of the first 100 cases (64 Anglo, 22 Spanish Am., 14 Indian) indicate the following: There appears to be earlier and more complete involution of the non-fat breast tissue in low risk ethnic groups after the age of early 20's. The presence of cystic change and ductal epithelial proliferation appears to be equally prevalent in all ethnic groups, increasing with age; radiologic interpretation of high risk patterns is occurring more often in the high risk groups; however, the radiologic interpretation does not seem to correlate well with presence of any of the putatively pre-malignant epithelial proliferative changes.
Computerization of the myriad of gross, histologic, radiologic and epidemiologic data is now under way.

Project Officers: Elizabeth P. Anderson, Ph.D.; Louise Brinton
Program: Breast Cancer Epidemiology
FY 80 Funds: $100,950

CONTRACT RESEARCH SUMMARY

Title: Effect of Neonatal Exposure to Estrogen and Progestin on the Mammary Gland

Principal Investigator: Dr. Jeffery M. Rosen
Performing Organization: Baylor College of Medicine
City and State: Houston, TX

Contract Number: NO1-CB-74189
Starting Date: 8/29/77 Expiration Date: 8/28/80

Goal: To study the influence of perinatal estrogen and progesterone administration on the development and expression of differentiated function in the murine mammary gland.

Approach: Newborn BALB/c mice will be injected with estrogen or progesterone or with both hormones. The organ cultured mammary glands will be exposed to: (a) insulin, hydrocortisone, and prolactin; (b) carcinogenic and non-carcinogenic analogues of DMBA; (c) thyroxine and growth hormone. Perinatal hormone treatment in virus-positive and negative BALB/c strains of mice will be compared. In addition, the effect of pretreatment with estradiol and progesterone for either 6 or 9 days at 4 or 5 weeks of age, prior to organ culture will be compared. Morphological and biochemical analysis including determinations of casein mRNA, and casein will be done.

Progress: Altered morphologic responses were observed after culture in tissue of BALB/c mice injected as newborns with estradiol or with estradiol and progesterone. Alveolar and lobular responses, as well as induction of casein mRNA were enhanced as the duration of pretreatment was increased from 6 to 9 with cortisol, prolactin and insulin in combination resulted in maximal alveolar and lobular development. In agreement with morphological data, perinatal estrogen administration resulted in an enhanced induction of casein mRNA in the presence of insulin, cortisol and prolactin, while perinatal progesterone treatment inhibited casein mRNA accumulation compared to untreated controls. Perinatal estrogen plus progesterone was without morphologic or biochemical effect on the parameters measured. A comparison of the prolactin dose-response *in vitro* suggested that exposure to estrogen perinatally sensitized the mammary tissue to the subsequent addition of prolactin in culture. Patterns of casein synthesis within mammary tissue sections are being evaluated using a highly specific fluorescent antibody technique. The prolactin dose response has been repeated in virus positive animals, with similar results.

These results indicate that perinatal estrogen may enhance hormone-regulated mammary differentiation; that perinatal progresterone may inhibit later development; and that both steroids in combination may exert antagonistic effects, perhaps by direct action at the tissue level.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Prevention of the Formation and Progression of Mammary Gland Preneoplastic Lesisons

Principal Investigator:	Dr. Richard C. Moon
Performing Organization:	IIT Research Institute
City and State:	Chicago, IL
Contract Number:	NO1-CB-74207
Starting Date:	8/29/77
Expiration Date:	8/28/80

Goal: To study the use of retinoids to prevent the development and/or progression of preneoplastic lesions of the mammary gland.

Approach: Experimental animals are fed non-toxic levels of synthetic vitamin A analogues (retinoids) as a dietary supplement in the attempt to inhibit the development and progression of preneoplastic lesions in the mammary glands of mice and carcinogen-treated rats. Endpoints monitored are: incidence of hyperplastic alveolar nodules (HAN) and "spontaneous" mammary tumors in C3H/He mice, and incidence, latency, and multiplicity of chemical carcinogen-induced mammary tumors in Sprague-Dawley rats. The effects of beginning retinoid administration at various times after carcinogen administration is being examined, as is the influence of retinoid administration combined with bilateral ovariectomy of carcinogen-treated animals.

Progress: Studies to evaluate the efficacy of retinyl acetate (RA), retinyl methyl ether, 4-hydroxyphenyl retinamide, retinyl butyl ether, 13-cis-retinoic acid, and axerophthene in the inhibition of HAN and mammary tumors in C3H/He mice are nearing completion. A study in which the beginning of RA administration was delayed for various periods after carcinogen administration to Sprague-Dawley rats is ongoing. Preliminary data indicates that retinoid administration can be delayed and retain its chemopreventive effect; the length of delay allowable without decreasing efficacy of inhibition is inversely related to carcinogen dose. A study in rats in which the first carcinogen-induced tumor is removed followed by RA treatment and/or ovariectomy (ovex) indicates that retinoid administration alone induces a slight delay in second tumor appearance. Ovex also increases the latency of the second tumor, while combined RA + ovex is most effective in delaying second tumor appearance. RA was found to selectively inhibit carcinogen induced increases in DNA synthesis in mammary parenchymal cells; retinoids inactive against mammary cancer have little effect on mammary DNA synthesis. Retinoic acid binding proteins (cRABP) in mammary tumor tissue were separated as 2S components using sucrose density gradient analysis. Relatively higher levels of cRABP were observed in ovarian hormone independent tumors compared to the ovarian hormone dependent tumors. Under appropriate conditions, [^{3}H]retinoic acid-cRABP complex translocates to the Nucleus.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: The Response of the Embryonic Mammary Gland to Androgenic Hormones

Principal Investigator: Dr. Klaus Kratochwil
Performing Organization: Institute für Molekularbiologie
City and Country: Salzburg, Austria

Contract Number: NO1-CB-33883
Starting Date: 6/29/73 Expiration Date: 3/28/80

Goal: To study the acquisition of hormone responsiveness during development of the mammary gland. Analysis of the androgen response with special emphasis on the role of tissue interaction.

Approach: Determine androgen-sensitive phase of mammary rudiment by exposing explanted mammary buds of various developmental stages to testosterone in vitro. Determine the target tissue for the hormone by experimental tissue combinations (gland epithelium and surrounding mesenchyme) taking advantage of the androgen-insensitive *Tfm*-mutant. Find out how the primary target tissue affects its partner (methods: Experimental tissue combinations, electron microscopic and histochemical study of the response itself, autoradiography of suitable precursors - especially if tissue interface material). Determine whether the duration of the androgen-sensitive phase of the gland is specified by transient hormone responsiveness of the (primary) target tissue or by changing properties of its partner (hormone binding in relation to responsive phase, combination of tissues in different developmental stages). Compare androgen response in different mammalian species.

Progress: Established that testosterone response in vitro is identical to the reaction in vivo. The temporary androgen-sensitive phase in mammary gland development was found to reflect intrinsic developmental changes in the gland itself. Established that the target tissue for testosterone is the mesenchyme and found that only the mesenchymal cells at the epithelial surface bind 3H-testosterone. Testosterone is bound before, during, and after the responsive "window". Found that several steps of cell and tissue interactions are required before and in the androgen response: At least 2 days before the hormone response mammary epithelium must act on neighboring mesenchymal cells. This epithelial influence is organ but not species-specific, its result is the appearance of testosterone receptors in adjacent mesenchymal cells. The later condensation of the mesenchyme can only be initiated by the cells at the epithelio-mesenchymal interface and it forms without participation of cells from distant sites. Finally, this testosterone-activated mesenchyme (but not the hormone itself) causes necrosis of the epithelium. Although it was found that this action of the mesenchyme on mammary epithelium involves degradation of the epithelial basal lamina, its mechanism is not known. The response of rat glands resembles that of mouse glands whereas the rudiments of another rodent (guinea pig) and a non-rodent (rabbit) are unresponsive to testosterone.

Project Officers: Chester V. Piczak, B.S., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Influence of Mammary Carcinoma Cells on the Formation and Absorption of Collagen

Principal Investigator: Dr. Gerhard Wirl
Performing Organization: Institute für Molekularbiologie
City and Country: Salzburg, Austria

Contract Number: NO1-CB-74109
Starting Date: 8/15/77 Expiration Date: 8/14/80

Goal: To isolate and characterize collagenase and inactive forms of collagenase present in mammary tumor tissue.

Approach: Studies are designed to: (a) extract collagenase directly from DMBA-induced tumors in the rat and spontaneous tumors in C_3H mice as well as from tumor tissue cultured media; (b) purify the enzyme and examine its purity using disc gel electrophoresis and immunodiffusion; (c) detect the activity of the enzyme; (d) determine the distribution of the enzyme in the tumor; (e) find any inactive form of the enzyme as a proenzyme; (f) determine the production of collagenase by epithelial cells or stromal cells. Studies of human tissues depend on results obtained from the mouse and rat.

Progress: The cell type responsible for the production of collagenase found in about one third of 65 DMBA-induced tumors of the rat was investigated. There is no evidence that cultures of tumor cells release collagenase. In contrast, macrophages isolated from these tumors have been found to secrete this enzyme in vitro. This has been demonstrated by cultures of stromal cells on plastic and on collagen gels. In most experiments this activity is enhanced by ingestion of latex and silica particles. The major part of the collagenase present in the culture fluids was in the active form with the latent enzyme able to be activated with trypsin. Our data suggest conversion of latent enzyme to active collagenase by neutral proteases secreted by the same cell fraction. Plasminogen-activator (PA) was found in the culture fluids from macrophages and from tumor cells. Whether stimulation of macrophages to produce collagenase and PA is mediated by factors released by tumor cells or by lymphocytes is now under study.

Other studies indicate that pronase digests of Walker 256 tumors also release collagenase into the culture media but the producer cell is still unclear. These tumors contain great amounts of procollagenase obviously bound to the collagen fibrils.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Role of Stroma in Growth of Neoplasms of the Mammary Gland

Principal Investigator: Dr. Merton R. Bernfield
Performing Organization: Leland Stanford Jr. University
City and State: Stanford, CA

Contract Number: NO1-CB-53903
Starting Date: 4/15/75 Expiration Date: 6/30/80

Goal: To define the role of the basal lamina, its constituent glycosaminoglycan (GAG) and the stroma in postnatal mammary development and neoplasia.

Approach: Elucidate the mechanisms underlying the assembly and maintenance of the basal lamina by normal cells and the lamina defects occurring in association with neoplastic invasion.

Progress: We have developed an in vitro system to compare normal Namru mouse mammary epithelial cells and their neoplastically transformed derivatives. Although no lamina is formed when the normal cells (< 20 passages) are cultured on plastic, when grown on a type I collagen gel, their rate of proteoglycan (PG) degradation is reduced and a PG-rich lamina is formed. When injected into nude mice, these cells form cysts encircled by a continuous lamina. Injection of high passage strains of this line results in pleiomorphic malignant tumors with only traces of a lamina at sites of local invasion. When these malignant cells are cultured on a collagen gel, only traces of a lamina are seen, duplicating the in vivo situation.

Whether on collagen (where only normal cells form a lamina), or on plastic (where neither cell line produces a lamina), the cell lines synthesize PGs at similar rates, produce PGs with both similar chemical composition (predominantly heparan sulfate PGs) and chromatographic distribution on Sepharose 4B, and regardless of substratum, secrete PGs into the medium at similar rates. In contrast with the normal cells, however, the malignant cells fail to respond to the collagen gel substratum with a reduced rate of PG degradation. Importantly, a qualitative difference is also found: the formation of a lamina by the normal cells is associated with the appearance of a unique PG peak on Sepharose 4B. Kinetic studies suggest that the unique peak is derived from an apparently larger, membrane-associated PG fraction. Cellular localization studies suggest that the putative precursor is exclusively intracellular, while the unique peak is a component of the basal lamina.

Taken together, the data suggest the working hypotheses (a) that collagen normally facilitates the conversion by the mammary epithelial cells of a precursor PG into a PG fraction which resists degradation because it assembles as part of the lamina, and (b) that the malignant mammary cells are defective in forming a lamina because they fail to respond normally to the collagen substratum. This abnormality in the influence of stromal collagen could allow invasive growth.

Project Officers: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Growth and Passage of Primary Culture of Normal Mammary Epithelial Cells

Principal Investigator: Dr. Dayton S. Misfeldt
Performing Organization: Leland Stanford Jr. University
City and State: Stanford, CA

Contract Numer: NO1-CB-74094
Starting Date: 7/1/77 Expiration Date: 6/30/81

Goal: To define conditions for the growth and passage of normal mammary epithelial cells in culture.

Approach: Conditions for growth and passage are determined by colony and cell count with an established mouse mammary cell line (NMUMG) and primary cultures of Balb/c mouse mammary gland.

Progress: Utilizing the established mammary cell line, it has been determined that cloning efficiency and growth is optimal at pH 7.0-7.2. Further, an atmosphere of 5% CO_2 at the same time pH in media buffered with bicarbonate and HEPES increases growth and cloning efficiency over ambient conditions. Similar pH optima were demonstrated for primary culture of mouse mammary gland.

The usefulness of D-valine in suppressing fibroblast growth in primary culture is nullified by a marked inhibition on the growth and cloning efficiency of NMUMG and primary culture.

The effectiveness of early passage of primary culture has been improved by the addition of methyl cellulose to trypsin, EDTA dissociation treatment. Fibroblast overgrowth has been managed by brief trypsin exposure and wash which removed the rapidly released fibroblasts. Five cell lines from three separate dissociations have been prepared. All are epitheliod in appearance and one is in the ninth passage. Presently, the following collaborative studies are in progress to define the extent to which phenotypis features of mammary epithelia are retained; these include karyology, outgrowth patterns in cleared mammary fat pad in Balb/c and CfZ (for susceptibility to MTV infection), reactivity to milk-fat globule membrane and Thy 1.2 antibody.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: $79,600

CONTRACT RESEARCH SUMMARY

Title: Animal and Human Mammary Tumors and Human Cell Culture Bank Facility

Principal Investigator:	Dr. Arthur E. Bogden
Performing Organization:	Mason Research Institute
City and State:	Worcester, MA
Contract Number:	NO1-CB-74175
Starting Date: 6/28/79	Expiration Date: 6/27/80

Goal: To function as a service facility for the cryopreservation, storage and distribution of biologically characterized and monitored (a) animal and human mammary and endocrine related tumors transplantable in vivo, and (b) cell lines of human and animal breast tumor origin established in in vitro culture, for use by the BCTF and other selected investigators.

Approach: Cryopreservation of mammary and endocrine related tumors of animal and human origin, as well as human breast tumor cell lines: (a) submittal of tumors and cell lines by qualified investigators able to furnish pertinent background information; (b) biological characterization of transplantable tissues, both pre- and post-cryopreservation, by determination of growth curves, specific effects on host organs, host (syngeneic and xenogeneic) survival, serum hormone levels, histology, karyology, response to ablative procedures; (c) cell lines tested for viability, plating efficiency, freedom from contamination and tumorigenicity by nude mouse implantation; (d) characterized tissue and cell lines preserved in Linde liquid nitrogen freezers according to well recognized, proven procedures.

Progress: During the past contract period 149 tumors were shipped to 83 investigators; 55% were of mammary and 6% of anterior pituitary origin. Thirteen new tumors were received for cryopreservation and characterization and 66 Noble tumors (non-mammary) were transferred to the DCT sponsored Tumor Bank, resulting in a current inventory of 259 different transplantable tumors or sublines stored in 5,851 ampules in liquid nitrogen. Five human breast tumors, transplantable in athymic nude mice, are now cryopreserved and retrievable for distribution. Human and rat α-lactalbumin with specific antisera have been shipped to three investigators. Four new human breast tumor and three normal epithelial cell lines have been received for expansion and cryopreservation resulting in a current inventory of 20 human breast tumor, four human normal epithelial, and two mouse and one rat mammary tumor cell lines in cryopreservation. Sixty-two shipments (21 as frozen ampules and 227 as viable cultures) were made to 51 different laboratories. In vivo tumorigenicity of all human cell lines are being determined by implantation into athymic nude mice. Estrogen and progesterone profiles are being established on the human breast tumor cell lines. A bibliography relative to transplantable tumors in the bank has been compiled and is available on request.

Project Officers: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: $154,000

CONTRACT RESEARCH SUMMARY

Title: Identification of Mammary Tissues

Principal Investigator: Dr. Melvyn S. Soloff
Performing Organization: Medical College of Ohio
City and State: Toledo, OH

Contract Number: NO1-CB-63983
Starting Date: 6/30/76 Expiration Date: 6/29/80

Goal: To isolate, identify, characterize and quantitate myoepithelial cells in rat mammary tissue and tumors of rat and human origin.

Approach: Separate myoepithelial cells by centrifugation in albumin gradients, using oxytocin binding, and scanning/transmission electron microscopy to identify the cells. Examine the potential of using alkaline phosphatase and ATPase as cell markers. Prepare specific antiserum and identify, quantitate and characterize myoepithelial cells in mammary glands from virgin, pregnant and postpartum rats and rat mammary tumors induced with DMBA. Parallel investigations will be carried out with human tumors obtained from surgery.

Progress: Isolated myoepithelial and secretory cells were purified from the mammary gland of the lactating rat. The two cell types could be distinguished morphologically by optical, TEM and SEM microscopy. Myoepithelial cells stained cytochemically for alkaline phosphatase activity. Isolated myoepithelial cells possess at least 10 times more p-nitrophenylphosphatase activity, Na, K-ATPase activity and high affinity sites for [^{3}H]oxytocin than secretory cells. The secretory cells contained glucose-6-phosphate dehydrogenase activity, which was not detectable in myoepithelial cells. Specific antisera to myoepithelial cells were produced in rabbits and can be demonstrated by peroxidase-anti-peroxidase staining of frozen sections of rat mammary tissues and isolated cells. [^{3}H]-oxytocin binding and alkaline phosphatase activities were studied as markers for mammary myoepithelial cells from the onset of pregnancy, through parturition and in lacation. Both activities were lowest at the beginning of pregnancy, increased steadily during pregnancy and were maximal during lactation. Glucose-6-phosphate dehydrogenase activity fell to baseline levels by 48 hours after weaning whereas alkaline phosphatase and the concentration of oxytocin receptors remained elevated for at least 6 days in the involuting mammary gland. A 700-fold purification of alkaline phosphatase from myoepithelial cells was achieved. Twenty-seven human breast samples were assayed for [^{3}H]oxytocin binding activity and binding was more substantial in fibroadenomas and in some samples of fibrocystic disease. Binding was negligible in adenocarcinomas of the breast. This may indicate that myoepithelial cells may be involved in the pathogenesis of sclerosing adenosis and may become prominent in fibroadenomas.

Project Officer: Chester V. Piczak, B.S., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Prevent the Formation and Progression of Mammary Gland Preneoplastic Lesions

Principal Investigator: Dr. Clifford W. Welsch
Performing Organization: Michigan State University
City and State: East Lansing, MI

Contract Number: NO1-CB-74090
Starting Date: 8/29/77 Expiration Date: 8/28/80

Goal: To prevent the early development of preneoplastic mammary gland lesions and progression of these preneoplastic lesions to neoplastic lesions by chronic suppression of pituitary prolactin secretion.

Approach: A prolactin-suppressing drug, 2-bromo-α-ergocryptine (CB-154) will be administered prior to and after the spontaneous development of, or the induction of mammary gland dysplasias. The following mammary gland dysplasias will be examined: A: Mice: 1) spontaneously occurring alveolar lesions (HAN) - DBA/2; 2) pregnancy-induced alveolar lesions (HAN) - A/J; 3) DMBA-induced lesions (DH) - Balb/c; 4) estrogen and progesterone induced and pregnancy-induced ductal lesions (plaques) - GR/A. B. Rats: 1) DMBA-induced ductal and alveolar lesions - Lewis, Long-Evans and Sprague-Dawley; 2) X-irradiation-induced ductal and alveolar lesions - Sprague-Dawley; 3) NMU-induced ductal and alveolar lesions - Sprague-Dawley. C. Retinyl acetate feeding will be compared with prolactin suppression on the genesis of mammary dysplasias in NMU treated Sprague-Dawley rats and estrogen-progesterone treated GR/A female mice. D. Tamoxifin (an estrogen antagonist) will be compared with prolactin suppression on the genesis of mammary dysplasias in DMBA treated and X-irradiated Sprague-Dawley rats.

Progress: The suppression of prolactin secretion with CB-154 has markedly reduced the incidence of mammary gland dysplasias in carcinogen (DMBA and NMU) treated female Sprague-Dawley rats and carcinogen (DMBA) treated female Lewis and Long-Evans rats. Suppression of prolactin secretion has also sharply reduced the incidence of pregnancy and estrogen-progesterone induced mammary gland dysplasias in female GR/A mice. Suppression of prolactin secretion does not influence the development of mammary gland dysplasias in carcinogen-treated female Balb/c mice. Prolactin suppression appears to be just as effective as retinyl acetate feeding in the prophylaxis of NMU induced mammary tumorigenesis in female Sprague-Dawley rats. A combination of prolactin suppression and retinyl acetate feeding is far superior than either treatment alone in the prophylaxis of NMU-induced mammary tumorigenesis in female Sprague-Dawley rats. Prolactin suppression and Tamoxifin treatment reduces the incidence of irradiation induced mammary tumorigenesis in female Sprague-Dawley rats. It also appears that the dose of the carcinogen markedly influences the effectiveness of prolactin suppression, i.e., in low doses of administered carcinogens (DMBA), prolactin suppression appears to be more effective in prophylaxis of murine mammary tumorigenesis.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Metabolism of Polycyclic Aromatic Hydrocarbons in the Induction of Mammary Tumor

Principal Investigator:	Dr. A. Monaem El-hawari
Performing Organization:	Midwest Research Institute
City and State:	Kansas City, MO
Contract Number:	NO1-CB-84227
Starting Date:	9/1/79
Expiration Date:	8/31/81

Goal: To determine whether differences in the disposition, metabolism and macromolecular binding are related to differences in mammary tumor incidence caused by polycyclic aromatic hydrocarbons.

Approach: Carcinogenesis studies are being performed in virgin female rats (≈ 50 days old) from Sprague-Dawley (SD), Long-Evans (LE) and Wistar-Lewis (WL) strains treated with 3-MC, 7,12-DMBA or vehicle (oil). In this study, sexual maturity of rats was confirmed before dosing. In addition, disposition, metabolic and binding studies are being performed in vivo and in vitro in these strains, in male SD rats and in younger, older, pregnant and lactating rats from the SD strain.

Progress: Carcinogenesis studies confirmed the high susceptibility of SD rats to mammary tumor induction by 7,12-DMBA. WL rats also showed high carcinogenic index, but LE rats were less susceptible. After treatment with 3-MC, WL rats were the most susceptible while LE rats were the least susceptible. Disposition and metabolic studies suggested that the low response of LE strain was not due to the inability of this strain to absorb and transmit the carcinogens to the mammary gland. Metabolic profiles in tissues and excreta are being analyzed for qualitative and quantitative differences. Studies in pregnant and lactating rats indicated that considerable amounts of both carcinogens were transported through the placenta and eliminated in the milk. Alterations in metabolism of the carcinogens due to pregnancy and lactation are being assessed. In vitro metabolic studies showed that both carcinogens were metabolized by mammary gland microsomal preparations. Preliminary HPLC analysis suggested that some metabolic products differ from those produced by the liver. Aryl hydrocarbon hydroxylase in the mammary gland was inducible but epoxide hydrase was not. Methods are under development for metabolic and binding studies in intact cells from liver and mammary gland.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: $144,000

CONTRACT RESEARCH SUMMARY

Title: Produce and Identify Antibodies to Collagens/Procollagens and/or Related Enzymes

Principal Investigator: Dr. Burton Goldberg
Performing Organization: New York University Medical Center
City and State: New York, NY

Contract Number: NO1-CB-84314
Starting Date: 9/1/78 Expiration Date: 8/31/81

Goal: To produce specific antibodies to the carboxyterminal non-helical sequences of human type I procollagen in sufficient quantities for storage and distribution to other investigators.

Approach: Culture human skin fibroblasts (CRL1121) in roller bottles and isolate the procollagen in the culture medium then subject it to specific bacterial collagenase digestion. Purify the carboxyterminal fragment by ion exchange chromatography then produce antibodies in appropriate animal species (rabbit, goat, sheep). Purify the antisera by absorption techniques and/or affinity chromatography and confirm their specificity by Ouchterlony technique, immunoelectrophoresis, passive hemaglutination and radioimmunoassay.

Progress: The tissue culture facilities provided under the contract have permitted the collection to date of 120 liters of medium from confluent cultures of human skin fibroblasts. Approximately 1 gram of procollagen-enriched lyophilate has been harvested from these collections and a portion of that material subjected to collagenase digestion to generate the nonhelical carboxy-terminal propeptide fragment from procollagen. The propeptide fragment has been purified by ion exchange chromatography on DEAE-cellulose and to date 65 mg. of the pure protein has been prepared. A portion of the protein has been committed to immunization protocols in 7 rabbits and one sheep. The antisera from the animals give single precipitin lines when tested against the antigen in Ouchterlony plates. A specific radioimmunoassay has been used to titer the developed antisera and to date some rabbit antisera have titers as high as 1/80,000. The sheep antiserum currently has a titer of 1/10,000. The boosting schedules are still in progress and it is anticipated that the test bleed volumes will soon be increased for the sheep and the rabbits.

Some of the propeptide antigen has been coupled to cyanogen bromide activated Sepharose 4B with the goal of purifying the antisera by affinity chromatography. The rabbit antisera will be used in pilot studies of affinity purification and the sheep antisera will then be committed to the procedure. They anticipate that affinity-purified antibodies will become available by September 1980.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: $71,325 Terminal

CONTRACT RESEARCH SUMMARY

Title: Development of an Assay for Genetic Damage to Mammary Gland Cells

Principal Investigator: Dr. Steven D'Ambrosio
Performing Organization: Ohio State University
City and State: Columbus, OH

Contract Number: NO1-CB-84226
Starting Date: 9/1/78 Expiration Date: 8/31/81

Goal: To develop an inexpensive, rapid and quantitative assay for detecting genetic damage and its repair in mammary cells in vivo following exposure to carcinogenic agents.

Approach: Measure DNA damage directly by an assay using S_1 endonuclease. Determine changes in the size of non-radiolabeled DNA resulting from endonuclease treatment using alkaline or neutral sucrose gradient sedimentation. After sedimentation, the non-radiolabeled DNA will be quantitated by a spectrofluorometric technique. Quantitate the mammary epithelial cell DNA damage by double label autoradiography. DMBA sensitive and resistant rat strains and non-carcinogenic and less carcinogenic analogs 2-FL-DMBA and 5FL-DMBA will be used for these studies.

Progress: Initial studies on binding of ^{3}H-DMBA to rat mammary DNA has been completed. Maximum binding occurred at 24 hours with a slight decrease after 7 days. These data also showed that a large amount of the radioactive DMBA was non co-valently associated with the DNA. This association is probably due to a strong intercalation of DMBA into duplex DNA. The binding experiments were repeated using higher specific activity DMBA. Hopefully, this increase activity will allow adduct characterization. Liver, lung, kidney, and heart will be analyzed to compare the initial and long term binding of DMBA to their DNA with that of mammary DNA. ^{3}H-2-fluoro-DMBA will be obtained to initiate studies using this analog of DMBA to allow assessment and comparison of the extent of binding of this presumed non-carcinogenic analog with the carcinogenic DMBA. Studies were initiated to detect by fluorescence the metabolites produced by DMBA and its 2 and 5 fluoro analogs in order to determine whether metabolites (types and amounts) can be used as indicators of exposures to poly aromatic hydrocarbon carcinogens. Metabolites were collected and are being analyzed to compare the number of tumors produced to assess the predicatability of this assay in terms of carcinogenesis.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: $134,579

CONTRACT RESEARCH SUMMARY

Title: Change in Mammary Gland Sensitivity to Carcinogens Related to Host Physiological State

Principal Investigator: Dr. Dilip K. Sinha
Performing Organization: Roswell Park Memorial Institute
City and State: Buffalo, NY

Contract Number: NO1-CB-74105
Starting Date: 8/29/77 Expiration Date: 8/28/80

Goal: To investigate the susceptibility of the mammary gland to neoplastic transformation during changing conditions of the host due to age and pregnancy.

Approach: Study the DNA synthesis of ductal and alveolar cells of prepubertal, pubertal and postpubertal rats. Attempt to induce growth in mitotically static old mammary glands by transplantation. Determine the proliferative pattern of "old" and "young" mammary glands transplanted into the gland-free fat pad. Attempt to induce tumors in old mammary glands transplanted into the gland-free mammary fat pads of old rats.

Progress: The growth and development of the mammary gland in prepubertal, pubertal and postpubertal (aging) rats has been studied. Ten, 15, 25, 35, 55, 70, 90, 150 and 250 day old female rats were used in this investigation. The developmental pattern was established by studying wholemount preparations of the abdomino-inguinal gland and by DNA synthetic index as indicated by ^{3}H-thymidine uptake (labeling index). Mammary glands in 10 day old rats start as a single, branched duct with club-shaped end buds, which with age develop into multiple ductal system and ultimately almost fill up the fat pad. The proliferative index reaches its peak in 25 and 35 days; it then starts to decline. At 150 days and after, the glands are almost at stasis, considering proliferation. The second approach of this project was to investigate the proliferation and growth of pieces of mammary glands transplanted into isologous hosts. Small pieces of mammary tissue from 60-day-old rats were transplanted into the gland-free fat pad of 200 day old rats and vice versa. Graft-bearing rats were killed at 60, 90, 120 and 150 days after transplantation. Also, dissociated mammary cells from 200-day-old female rats were injected into the fat pads of isologous hosts. Both the small pieces and the dissociated cells grew to form a full ductal mammary structure. ^{3}H-thymidine labeling indices of these outgrowths were comparable to mammary glands from 50-60-day-old virgin female rats. Tumorigenesis in these outgrowths by 7,12-DMBA was tested. Preliminary data show that tumors can be induced in these outgrowths, irrespective of the age of the donor of the transplanted cells. Tumorigenesis was highest when DMBA was given 30 days after the mammary cell transplant. At this stage the ^{3}H-thymidine labeling index was also high (19%). More data are now being complied.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: · 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Morphological Properties of Normal and Abnormal Human and Rodent Mammary Tissue

Principal Investigator: Dr. Elinor Spring-Mills
Performing Organization: State University of New York
City and State: Syracuse, NY

Contract Number: NO1-CB-84239
Starting Date: 8/14/78 Expiration Date: 8/13/81

Goal: To evaluate structural differences among normal, dysplastic and cancerous mammary glands.

Approach: To describe, quantitate and compare certain morphological and functional characteristics of normal, hyperplastic and carcinomatous human and mouse mammary glands in vivo and in vitro. Human tissues obtained at biopsy, mastectomy and reduction mammoplasty will be prepared for light microscopy (LM), transmission electron microscopy (TEM), scan electron microscopy (SEM) and quantitative electron microscopy (QEM) or morphometry. Animal tissue from non-pregnant (pubertal and adult), pregnant, and multiparous C_3H mice will be studied.

Progress: Since June 1979 when all organ culture equipment was installed, tumors and proliferative lesions from 30 women have been explanted. Survival of 8 fibroadenomas, 6 benign mammary dysplasias, 4 fibrosing mastopathies, 9 invasive ductal carcinomas, 1 low grade papillary adenocarcinoma and 1 infiltrating lobular carcinoma has been followed in medium 199 without serum for 6-42 days. The effects of no hormones, insulin, hydrocortisone, prolactin and vitamin A alone and in combination have been tested. The results in vitro are being analyzed in relationship to the endocrine profiles and case histories of the women as well as the zero time morphology of the explanted tissues. Systems to support longer term (3-4 month) survival of human breast tissues are being developed with and without serum supplements.

The mouse project is well underway. It has become obvious that precursor lesions related to the HAN and other lesions of undetermined biologic significance are appearing in 2 1/2 - 3 month old C_3H/HeJ mice. The breasts, ovaries, adrenals, uteri and oviducts from 1 and 3 month old C_3H/HeJ mice have been studied with the LM, SEM and TEM. To more completely evaluate the morphologic findings, parallel studies are now being conducted in the C_3HeB/Fe mice which have low mammary tumor incidence.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: $80,869 Terminal

CONTRACT RESEARCH SUMMARY

Title: Influence of Carcinogens on Viral Gene Expression Leading to Mammary Cancer

Principal Investigator: Dr. Asher Frensdorff
Performing Organization: Tel Aviv University
City and Country: Tel Aviv, Israel

Contract Number: NO1-CB-74118
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To study longitudinally the influence of chemical carcinogens in combination with prolonged hormonal stimulation on murine mammary tumor virus-gene expression.

Approach: A) MuMTV gene expression is studied by measuring longitudinally, at different times following oncogenic stimulus, two parameters: 1) levels of the MuMTV antigens gp52 and p28 in mammary tumors in mammary gland tissues and in serum and milk of treated and control mice, 2) autogenous humoral immune response to MuMTV. Oncogenic stimulus will be DMBA, with pituitary isograft. A well defined colony of BALB/c mice, specially generated for this purpose to insure maximum homogeneity to spontaneous MuMTV expression is used. B) The controversial question wheter complete, or as has recently been suggested, uncoordinate-MuMTV gene expression is at all triggered in BALB/c mice by treatment with hormones and DMBA and more importantly, whether such possibly very transient expression, is a necessary step in the inducing of chemical carcinogenesis was approached as follows: Isografted and DMBA-treated BALB/c mice of our special colony (BALB/cTau) were either passively immunized against MuMTV by administration of anti-MuMTV antibodies or actively immunized with infective or inactivated MuMTV. All immunizations were given at different times relative to DMBA. The isograft-DMBA protocol they used differed in several important points from those used by other investigators, (e.g.: timing of isografting and DMBA treatment; DMBA was given by a single mammary skin painting; observation periods extended far beyond those of others).

Progress: This protocol leads to an unusually high tumor incidence, most, if not all of which are mammary (80% and 95% at 11 and 14 months after DMBA, respectively). At 17 months after DMBA, even mice only isografted displayed a high tumor incidence, whereas in totally untreated mice of our colony, so far spontaneous tumor incidence is nil. Anti-MuMTV given simultaneously with DMBA caused a delay in tumor appearance as compared to control groups, though eventually the final tumor incidence was unaffected. On the other hand, anti-MuMTV given after DMBA treatment, greatly enhanced tumor appearance. Some enhancement was also observed in mice actively immunized with MuMTV or with Moloney leukemia virus. Autogenous anti-MuMTV antibodies were monitored in all mice individually during the experiment. These observations provide a reproducible model for induction of high incidence of mammary adenocarcinomas. More importantly, they support the concept that MuMTV gene expression has an active role in prolactin plus DMBA induced mammary tumorigenesis. A better understanding of the mechanisms of action of DMBA will be obtained to determine whether or not carcinogen induced oncogenesis is dependent on viral gene expression.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Differentiation of Mammary Epithelial Cells

Principal Investigator: Dr. Howard L. Hosick
Performing Organization: Washington State University
City and State: Pullman, WA

Contract Number: NO1-CB-63986
Starting Date: 6/30/76 Expiration Date: 6/29/80

Goal: To study how several mesenchymal cell types in the adult mouse mammary gland interact with mammary epithelium to regulate its development.

Approach: Isolate mammary epithelial cells from C3H mice in midpregnant state. Grow these cells as monolayers in culture dishes and on various permeable substrata. Test the responses of the epithelium, under various endocrine conditions, to other mammary cell types and their products. The emphasis is on interactions with adipose cells and fibroblasts. Responses of epithelium to be measured in terms of growth and of differentiation (production of mammary-specific products). Responses of normal, preneoplastic, and neoplastic cells will be compared. Undertake studies for defining conditions for growth and passage of primary cultures of normal and premalignant epithelial cells.

Progress: A. Much of their effort has been focused on inserting unesterified fatty acids which might be liberated from mammary adipose tissues into mammary epithelial cells in culture. In one approach, liposomes have been constructed containing oleic, linoleic, or arachidonic acid. When these liposomes are added to culture medium, they fuse with apical cell surfaces, fatty acids enter the cells, and they are metabolized. Oleic acid so supplied enhances replication of midpregnant mammary cells by approximately 50%. Arachidonic acid is also marginally stimulatory and linoleic acid has some slight effect on growth. Also, cells have been grown on permeable substrata through which unesterified fatty acids can percolate to the basal surface of cells to be uptaken by the cells. Four such substrata have been tested: hollow fiber membranes, diethylaminoethyl chloride-coated Sephadex beads, hydrated collagen gels, and thin layers of collagen supported on nylon mesh. Cells have also been grown as clusters inside collagen-gel matrices; tubules grow out from such clusters, which superficially resemble mammary ducts in vitro. Growth properties of 'ducts' are being defined. B. Our chromatographic procedure for isolating small quantities of newly-made lactose from culture fluids has been improved. The assay has been used to measure lactose production by as few as 10^6 lactating cells. The assay is to be employed as a marker of differentiation. C. Optimal culture conditions for mouse and human mammary cells are being devised. Embryonic extract improves the quality (epithelial) morphology, replication rate of mouse cells, but as yet has not been effective on human cells isolated from milk. When lipids are removed from the extract it loses its growth-promoting ability.

Project Officers: Chester V. Piczak, B.S., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Produce and Identify Antibodies to Collagens/Procollagens and/or Related Enzymes

Principal Investigator: Dr. Heinz Furthmayr
Performing Organization: Yale University
City and State: New Haven, CT

Contract Number: NO1-CB-84225
Starting Date: 9/18/78 Expiration Date: 9/17/81

Goal: To prepare immunochemically defined antibody reagents to collagen of types I,II,III,IV,V, procollagen of types I, III and mouse collagen of types I, III.

Approach: Studies include: 1) isolation and purification of collagens from calf, human and mouse tissue for immunization, affinity columns and serological testing; 2) immunization of rabbits, sheep and mice; 3) serological screening of antisera during immunization and after final bleeding; 4) isolation and purification of antibodies specific for each collagen; 5) characterization of the isolated antibodies, and 6) storage and distribution of the purified antibodies to other investigators.

Progress: Various amounts of different types of collagen were isolated such as sufficient amounts of type I and type II collagen from calf; 2 gms of type I, 2 gms of type III and 70 mgs type V (AB_2) from human; 500 mg of type I and 400 mg of type III from lathyritic mouse skin; 500 gms of the EHS sarcoma grown in mice and stored frozen at -70°C. Isolation of chemically distinct species of basement membrane collagen ("7S" collagen [Timpl] and "Intima"-type [Miller]) was initiated. Groups of approximately 10 rabbits were immunized with calf type I, II: human type III, V; mouse type I, III and 4 rabbits were immunized with mouse IgG isolated from serum. Antibodies to type I, II and III were isolated and characterized by passive hemagglutination and immunofluorescence on tissue sections. Affinity columns were prepared for isolation of mouse type I and III, collagen antibodies and columns of rabbit and mouse IgG were prepared to isolate IgG antibodies for immunofluorescence, ultrastructural and antibody precipitation studies. Various strains of mice were tested for antibody response to mouse type IV and human type III and V collagen to determine high and low responder strains. While mouse type IV (EHS sarcoma) is not immunogenic, various haplotypes were identified which determine a high antibody response to type III and V collagen. Type IV collagen (pepsinized) from human placental membranes and villous portion and type V (placental membranes) were used to immunize SW mice and spleen cells obtained from immune mice were fused to SP-1 tumor cells to produce monoclonal antibody cells. This technique will be extensively pursued for the entire project period. The initial success indicates that monoclonal antibody reagents will provide valuable tools to study basement membrane collagens. Various techniques were developed: the ELISA assay for type IV collagen, an ultrathin frozen sectioning method for electron microscopic localization of collagen and other matrix glycoproteins (fibronectin, laminin). A rotary shadowing method was established which is invaluable for studies of the molecular structure of basement membrane collagen and non-collagenous matrix-molecules. Immunofluorescence technology is being applied to study the fibrotic response in human disease (lung), tumors, carbon tetrachloride induced experimental cirrhosis (mice) and schistosomiasis (mice).

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: $87,100

CONTRACT RESEARCH SUMMARY

Title: Purification of Estrogen Receptor in Tangible Amounts

Principal Investigator: Dr. Giovanni Alfredo Puca
Performing Organization: Universita di Napoli
City and Country: 80138 Napoli, Italy

Contract Number: NO1-CB-64074
Starting Date: 9/30/76 Expiration Date: 5/29/81

Goal: To isolate and purify the "native" form of the estrogen receptor in milligram quantities.

Approach: Carry out large scale tissue disintegration and centrifugation for isolation of estrogen receptor from calves uteri. Purify the "native" form of estradiol receptor by a combination of affinity chromatography on new adsorbents and conventional separation methods.

Progress: An easy and fast procedure for the purification of the native form of estradiol receptor from calf uterus has been achieved. After addition of 5 mmol $MgCl_2$ the high speed supernatant is incubated batchwise with agarose to which heparin has been covalently bound. Elution of the estradiol binding activity is obtained by heparin. The volume of the eluate is reduced to one-tenth the volume of the original cytosol and presence of heparin prevents the aggregation of the receptor. Immobilization of receptor on 17β-estradiol-17-hemisuccinyl-hexane agarose is best carried out on column. The washing of the specific adsorbent is very critical and it must be extensive. Elution is performed with chaotropic salt, NaSCN, in the presence of low concentration of estradiol. A final procedure requires elimination of some residual contaminating proteins. Sephadex G-200 chromatography in low salt buffer gives good results. Even after complete purification, native receptor maintains the tendency to aggregate in very large forms and these do not penetrate the polyacrylamide gel in the absence of sodium dodecyl sulphate. These are eluted in the void volume of Sephadex G-200 columns and very often sediment at the bottom of sucrose gradients. Presence of $mgCl_2$ cations during the purification procedure decreases the aggregation phenomenon.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Detection and Quantitation of Transformed Mammary Epithelial Cells In Vitro

Principal Investigator: Dr. William Niedermeier
Performing Organization: University of Alabama
City and State: Birmingham, AL

Contract Number: NO1-CB-74092
Starting Date: 8/29/77 Expiration Date: 8/28/80

Goal: The identification and isolation of mammary tumor related antigens for use in biochemical assays to discriminate between normal and neoplastic human mammary epithelium.

Approach: Human breast adenocarcinomas are being analyzed for presence of antigens that are related to mouse mammary tumor virus (MMTV). The antigen is identified by immunofluorescence, and isolated by immunospecific affinity chromatography. Physicochemical properties, including carbohydrate and amino acid compositions, will be determined.

Progress: Antibodies were detected by indirect immunofluorescence in blood serum of 41% of patients with adenocarcinoma of the breast, that reacted with antigenic components in frozen sections of BALB/cf C_3H mouse mammary tumors. The sera that were positive when tested against mouse mammary tumors were also positive when tested by direct immunofluorescence against the patients own tumor or against tumors of other seropositive patients with breast cancer. The immunofluorescence reaction could be blocked by absorption of the sera with mouse mammary tumor virus. Efforts to characterize the antigenic component toward which the serum antibody is directed, indicate that it is present both in the cell membranes and the cytoplasm. It appears to be a protein that is destroyed by trypsin, which is bound to RNA as judged from a marked increase in fluorescence intensity following treatment with ribonuclease.

They are attempting to develop a radioimmunoprecipitation assay (RIP) quantitation of the antibody. Whole lysed virus is labeled with ^{125}I and the immunoprecipitate that results when serum from breast cancer patients is added, is counted in a gamma counter. The results obtained by immunofluorescence have been confirmed as being either positive or negative, but quantitation is poor due to high background counts, presumably due to the use of crude antigen preparations. Experiments are in progress to identify and isolate the specific antigen for use in the RIP assay and for purposes of physicochemical characterization.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Microcirculation and Molecular Transport in Mammary Carcinomas

Principal Investigators: Dr. Joseph F. Gross Dr. Marcos Intaglietta
Performing Organization: University of Arizona University of California
City and State: Tucson, AR San Diego, CA

Contract Number: NO1-CB-63981
Starting Date: 6/14/76 Expiration Date: 9/13/80

Goal: To study local blood velocity and intraluminal hydrostatic pressure in tumor capillaries and quantitate movement of materials across the endothelium of single vessels into extravascular tissue in tumor microcirculation.

Approach: Fit Fischer 344 rats with a modified algire access chamber containing mammary adenocarcinoma for analyses of microcirculatory parameters. Obtain quantitative morphological data by planimetric techniques. Determine blood flow by measuring velocity of red blood cells using dual slit technique. Determine capillary hydrostatic pressure by micropipette technique. Develop analytical models to characterize microcirculatory transport in tumor systems.

Progress: Red blood cell (RBC) velocity, vessel diameter and blood flow were determined in the microcirculation of the BA 1112 sarcoma at different levels of ambient temperature. At an environment temperature of 25°C, the RBC velocity was 1.10±0.52 mm/sec (S.D.) in terminal arterioles, 0.41±0.39 mm/sec in capillaries and 0.32±0.31 mm/sec in collecting venules. The arteriolar inflow through single vessels was $10.2 \pm 4.7 \times 10^{-5}$ ml/min. At an environment temperature of 35°C, RBC velocity was increased approximately four times in the terminal vascular bed. In both groups the arteriolar inflow was distributed similarly down to the capillary level. Under acute hyperthermic conditions of 41.3°C, there was a significant reduction in arteriolar inflow ($3.5 \pm 1.5 \times 10^{-5}$ ml/min) and in the number of perfused capillaries. These studies indicate that blood supply to mesenchymal tumors can be enhanced by elevating the environmental and local temperature to 35°C. Compared to room temperature conditions the arteriolar volume inflow does not appear to perfuse an increased number of capillaries. Acute hyperthermic temperatures reduced the vascularization and transparency only in the tumor tissue portion of the chamber. Pressure measurements were carried out in venous capillaries and vessels in a rat mammary adenocarcinoma using a servo-nulling pressure measuring method. Pressures ranged from 10 cm H_2O in venous capillaries to 5 cm H_2O in 20-35 μm diameter venules. The pressures were constant over the period of observation (5-10 minutes). There was no evidence of pulsatile pressure related to the action of the heart. The low pressures found in the microvessels of the tumor tissue are lower than those in similar vessels in the control, non-tumor area. Correlation of this data with anatomical data and flow measurements suggests that the low pressure is a consequence of the proliferation of the capillary and venous vasculature without an increase in the number of arteriolar vessels. Thus the increased perfusion presumed to be necessary for the increased tissue metabolism occurs as a result of vasodilation of arterioles and the decrease in post-arteriolar flow resistance.

Project Officers: Chester V. Piczak, B.S., Richard Knazek, M.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Growth and Passage of Primary Cultures of Normal Mammary Epithelial Cells

Principal Investigator: Dr. Gordon H. Sato
Performing Organization: University of California-San Diego
City and State: La Jolla, CA

Contract Number: NO1-CB-74188
Starting Date: 8/1/77 Expiration Date: 7/31/81

Goal: To isolate and maintain growth of pur normal mammary epithelial cells in culture.

Approach: Determine the hormonal and nutritional requirements of established animal and human mammary tumor cell lines. Proceed with similar studies on primary cultures of human mammary tumors obtained from autopsies and mastectomies. Determine the hormonal content, blood serum components, and growth factors (ovarian, nerve, epidermal, etc.) to prepare a defined media to maintain growth and passage of normal mammary epithelial cell lines.

Progress: MCF-7 cells in serum-free medium containing as supplements insulin, transferrin, epidermal growth factor, cold-insoluble globulin and prostaglandin F_2 alpha grow but do not attach and spread well on the culture dish, so that the characteristic epithelial cell shape seen in serum-containing medium is not maintained. Initially it was found that crude preparations of serum alpha globulins - Cohn fraction IV, fetuin, or a partially purified preparation of alpha-2 macroglobulin from human serum - could induce a morphological change to the epitheliod cell shape at concentrations of 50 to 500 µg/ml. This observation led them to test a commercial preparation of an alpha-1 protein reported to mediate spreading of Hela cells. This preparation was found to be active in the same way as the other alpha globulins, but was effective at 10-fold lower concentrations. A preliminary examination of the commercial preparation showed that it was rather heterogeneous on SDS polyacrylamide gels and the specific activity of different lots varied considerably, so they initiated isolating the spreading factor from human serum. By making several modifications of the original procedure, which is based on the affinity of the active material for glass beads, they can now quickly and cheaply make large amounts of preparations which are consistently more active and less heterogeneous than the commerical products. Upon SDS gel electrophoresis after reduction with mercaptoethanol their preparations contain a single major band which comprises roughly 80% of the total protein on the gel. The relative amount of this band increases in preparations of higher specific activity. The molecular weight of this band is estimated at between 70,000 and 80,000 and does not significantly change in the absence of reduction. Some low molecular weight protein (7,000-10,000) is present in the preparations, but attempts to isolate an active low molecular weight factor have been unsuccessful. The material is inactivated by boiling, incubation with trypsin or incubation at pH2, but remains active if incubated at Ph 10.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: $71,536

CONTRACT RESEARCH SUMMARY

Title: Effects of Neonatal Exposure to Estrogen and Progestin on the Mammary Gland

Principal Investigator: Dr. Frank J. Talamantes, Jr.
Performing Organization: University of California-Santa Cruz
City and State: Santa Cruz, CA

Contract Number: NO1-CB-74091
Starting Date: 8/1/77 Expiration Date: 7/31/80

Goal: Determine the effects of perinatal exposure to exogenous estrogens and progestins, singly and in combination, on mammary gland noduligenesis and tumorigenesis in female mice.

Approach: C3H (MTV+, NIV+) and C3Hf (MTV-, NIV+) female mice will be treated perinatally with diethylstilbestrol (DES), 17α-hydroxyprogesterone caproate (HPC), or Ovral. The incidence, types, and age of appearance of mammary gland dysplasias will be recorded to determine if noduligenesis and tumorigenesis depend on (1) the type and dose of hormone, (2) direct action of hormone on the gland or on alteration of systemic factors. Changes in mammary sensitivity to an oral contraceptive (Ovral) and a chemical carcinogen (DMBA) after perinatal hormone exposure and the effects of hormone administration to lactating mothers on noduligenesis and tumorigenesis in female offspring will also be determined.

Progress: Animals treated perinatally with DES, HPC, Ovral or oil vehicle were sacrificed monthly for 12 months for C3H mice and for 18 months for C3Hf mice. Mammary gland whole mounts from these animals are being examined histologically. Estradiol, progesterone, prolactin and growth hormone levels in serum from C3H animals sacrificed at 3, 6, and 9 months of age are being determined and correlated with mammary gland tumor incidence. C3H mice treated with DES or HPC perinatally were fed 0.1 mg or 1.0 mg DMBA at 3 and 3½ months of age and palpated weekly for mammary gland tumors until 10 months old. Neonatal DES treatment lowered the age of appearance of tumors and increased the number of animals who developed tumors. Neither tumor incidence nor age of appearance were affected by prenatal DES exposure. Tumor incidence was higher and the age of tumor appearance was lower in DMBA-treated mice who received DES, prenatally or neonatally, than in DMBA-treated mice who received oil perinatally. In contrast, neonatal or prenatal treatment with HPC did not affect tumor incidence or the age of tumor appearance or increase the sensitivity of the mammary gland to subsequent treatment with DMBA. The effects of perinatal DES and HPC treatment on mammary gland sensitivity to long-term Ovral administration are being determined.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Effect of Mammary Carcinoma Cells on Vascular Endothelium

Principal Investigator: Dr. Richard R. Lindquist
Performing Organization: University of Connecticut
City and State: Farmington, CT

Contract Number: NO1-CB-74089
Starting Date: 8/1/77 Expiration Date: 7/31/80

Goal: To elucidate the cancer cell-endothelium interaction and the effect cancer cells have on the vascular endothelium.

Approach: Human mammary cancer cells in co-culture with enodthelial cells will be the model in studies to determine: (a) cancer cell-endothelial interactions using time-lapse phase contrast microscopy and electron microscopy; (b) interacting forces involved; (c) influence of various blood elements and cellular organelles on strength of the interactions; (d) cancer cell inflicted damage to endothelium; (e) cancer cell induced directional proliferation of endothelial cells. Human cells will be obtained from the Cell Bank (BCTF) and endothelial cells will be isolated from umbilical cords.

Progress: Cinemicroscopy has revealed that cancer cells interact with and adhere to endothelial cells. The adhesion and rate of adhesion was quantified by using radio-labelled cancer cells. We find that the adhesion is prevented by cytochalsin B and thus indicates that microfilaments play an important role in the adhesion process. Our continuing cinemicroscopic studies have provided some new insights into how cancer cells after adhering to endothelium penetrate the endothelium and establish extravascular metastatic foci. We observed that some endothelial cells undergo lysis shortly after cancer cells adhere to them. Since we do not observe similar lysis of endothelial cells growing without added cancer cells we concluded that the lysis is inflicted by the cancer cell. Since some endothelial cells adjacent to cancer cells also undergo lysis we suspected that the cancer cell may release some soluble cytolytic product. In order to investigate this possibility we looked for soluble cytolytic products in the tumor cells. The cancer cells contain a small molecular weight (850-100) soluble cytoplasmic component which is highly lytic to certain target cells. The tumor cells also contain a large molecular weight (200,000) component which inhibits the lytic action of the samll molecular weight cytolytic component. Thus the tumor cells appear to have a protective mechanism which prevents the cytolytic component from inflicting self injury.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Methylation of Transfer RNA in Normal and Neoplastic Mammary Tissue

Principal Investigator: Dr. Phoebe S. Leboy
Performing Organization: University of Pennsylvania
City and State: Philadelphia, PA

Contract Number: NO1-CB-74108
Starting Date: 8/15/77
Expiration Date: 8/14/80

Goal: To examine tRNA methylation in normal mammary gland and DMBA-induced hormone responsive and non-responsive tumors in the rat.

Approach: Compare the level of methyltransferase activity in normal quiescent, late pregnant and lactating mammary tissue and DMBA-induced mammary tumors. Compare patterns of tRNA methyltransferase activities in hormone responsive and non-responsive tumors with those from non-neoplastic mammary tissue. Purify individual tRNA methyltransferases from mammary tumors and corresponding enzymes from normal mammary gland and compare their biochemical properties.

Progress: The total tRNA methyltransferase activity/mg tissue protein in tumor extracts is 3 times higher than in normal mammary extracts and about 40% higher than in extracts from late-pregnant mammary gland; however, the relative distribution of individual methyltransferases is the same in all three extracts. The extent of methylation of a limiting amount of tRNA substrate with normal mammary extracts is only 40-70% of that achieved with tumor enzymes. When analyzed with purified tRNAs, this difference appears attributable to an increased capacity of tumor extracts to approach the theoretical maximum of 1 methyl group/site/tRNA molecule in the formation of 5-methylcytosine and 2-methylguanine.

The 2-methylguanine methyltransferases from mammary tumors, normal mammary glands and glands from pregnant rats have been purified and their properties compared. The purified tumor enzyme responsible for 2-methylquanine formation at position 10 on tRNAs appears identical to that found in non-neoplastic tissues. In contrast, the tumor enzyme capable of methylating quanine at position 26 differs from its non-tumor counterpart in several respects. It shows altered chromatographic properties on DEAE-Sephadex and increased stability on isoelectric focusing. Most significantly, the tumor enzyme, unlike its normal counterpart, methylates several tRNAs which do not have an available quanine at position 26, including yeast $tRNA^{Phe}$ which is already methylated at positions 10 and 26. These results suggest that selected tumor methyltransferases may cause elevated extents of methylation because they aberrantly methylate novel sites on tRNA.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
FY 80 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator: Dr. Lewis M. Schiffer
Performing Organization: Allegheny-Singer Research Corporation
City and State: Pittsburgh, PA

Contract Number: NO1-CB-75083
Starting Date: 7/1/77 Expiration Date: 9/30/80

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: The experiments on two animal tumor models, the DMBA-induced rat mammary tumor and the C3H/He mouse spontaneous mammary tumor, will test the correlation of unperturbed tumor cell kinetics and tumor response in individual animals to chemotherapeutic agents. The hypothesis to be tested is that the highly variable cell kinetic parameters are related to the drug responsiveness of tumors. The cell kinetic studies will include tritiated thymidine labeling index, DNA synthesis time and tumor growth fraction estimation via primer-available DNA polymerase assay.

Progress: The basic cell kinetic studies on both tumors and biopsy material are complete. The data for the DMBA-induced rat primary tumor are complete and are being analyzed, and the cell kinetic data on the C3H/He primary tumor are also almost complete. In the rat-5FU studies we found that tumor regression was greatest in tumors with the highest PDP index, but regrowth delay did not correlate with cell kinetic values. In the rat-MTX studies the highest ^{3}HTdR LI tumors had the shortest time to nadir volume regression. Likewise, the rat-VCR studies showed that the highest ^{3}HTdR LI tumors had the greatest regression rate per day. The shorter the cell cycle time, the greater was the regrowth delay. Similar results were found in the rat-Adriamycin studies. In the rat-Cytoxan studies the cell kinetic values did not correlate well with tumor response, although when good responders were compared to poor responders it was found that in both groups, the PDP was a correlative factor. The mouse-Cytoxan studies, likewise, showed no simple correlative relationship between the cell kinetics and tumor response. The metastases part of the program is underway with studies in animals with and without primary tumors.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Therapy for Stage II and Stage III Carcinoma of the Breast

Principal Investigator: Dr. Charles A. Hubay
Performing Organization: Case Western Reserve University
City and State: Cleveland, OH

Contract Number: NO1-CB-43990
Starting Date: 6/16/74 Expiration Date: 6/15/81

Goal: This clinical trial was initiated in September, 1974, to determine if combined chemotherapy (CMF), anti-estrogen therapy (Tamoxifen) and immunotherapy (BCG) following mastectomy in Stage II, III breast cancer would yield better results than when used later in the course of the disease when recurrence became manifest.

Approach: Patients under age 76 who show axillary nodes involved with metastases at the time of surgery are eligible for this study. Stratification, but not treatment selection, was on the basis of the presence or absence of estrogen receptor protein (ER) in the tumor. Random treatment assignments were (1) cyclophosphamide, methotrexate, 5-fluourouracil (CMF); (2) cyclophosphamide, methotrexate, 5-fluourouracil, Tamoxifen (CMFT) and (3) CMFT for 12 months plus BCG for 12 months. Endpoint is the first evidence of treatment failure, i.e., the appearance of locally recurrent or of distant tumor.

Progress: This study was closed in June, 1979, after three hundred eighteen patients were enrolled. Of these, 76% had estrogen receptor (ER) positive tumors ($\geq$3 femtomoles/mg of protein). Of the treated patients, 40 have died and 74 have had treatment failure. Thirty-three patients have withdrawn voluntarily after one month to one year of therapy. Chemotherapy was not accompanied by significant myelosuppression. Hair loss was slight. Tamoxifen, the anti-estrogen agent used, was tolerated well, without serious side effects, although most patients experienced hot flashes while on the drug. Seventy-four patients have had recurrence: 30 in the CMF group, 25 in the CMF + Tamoxifen, and 19 in the CMF + Tamoxifen + BCG group. Statistical comparison for treatment failure between Stage II ER positive and ER negative patients is significant at the $p < 0.0001$ level, with the latter group recurring more rapidly. Relapse rate for the Stage II treated patients at 36 months is as follows: CMF - 39.4%, CMF + Tamoxifen - 37.2% and CMF + Tamoxifen + BCG 30.9%. Recurrence rates between Stage II patients with 1-3 positive nodes are significantly different when stratified according to estrogen receptor values, with higher relapse rates in the ER negative group.

Stratification by estrogen receptor assay shows a significant reduction in recurrence when Tamoxifen is added to CMF therapy in ER+ patients ($p=0.05$). There is no difference in recurrence rates for ER negative patients among the three treatment groups.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $74,700

CONTRACT RESEARCH SUMMARY

Title: Data Research Analysis for Breast Cancer Treatment Program

Principal Investigator: Ms. Marlene Dunsmore
Performing Organization: EG&G Mason Research Institute
City and State: Rockville, MD

Contract Number: NO1-CB-74140
Starting Date: 3/29/77 Expiration Date: 3/28/81

Goal: To increase the usefulness of data produced in projects related to the treatment of human breast cancer.

Approach: A central data file has been developed for three areas of study: (1) surgical adjuvant therapy; (2) tumor markers; and (3) estrogen receptor assays. The file allows comparisons of the results from various studies and provides a data base from which material can be quickly and conveniently retrieved. This data file is also implemented for testing new ideas, identifying groups of patients suitable for more detailed study, and for preparing reports to the medical community and the general public. The file is not intended to duplicate those at individual institutions but to perform analyses that are not possible at the individual institutions.

Progress: Mason personnel have designed a data collection and editing system for the Breast Cancer Studies Data Center. Clinical information is abstracted and entered via an IBM on-line disk file. The main file update system modifies clinical history files with new information and continues to edit for data consistency. Data concerned with treatment, histopathology, survival, and estrogen receptor status have been stored on 2,306 patients. Personnel have prepared extensive tabulations of these data for general meetings of the Breast Cancer Task Force Committee. They have sent each participating institution an analysis of its own data and of the collated information.

In February 1978 work was begun in support of the Biological Markers Project. The basic data collection and editing systems are operative. Background and clinical data have been gathered on more than 10,500 patients from three institutions. Mayo Clinic is serving as the repository for the serum samples. Benign tumor and metastatic cancer follow-up information is now being gathered. Since April 1979 serum specimens have been distributed to seven specified laboratories.

Project Officers: Mary E. Sears, M.D., Ihor Masnyk, Ph.D.
Program: Breast Cancer Treatment
FY 80 Funds: $127,734

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II or III Carcinoma of the Breast

Principal Investigator:	Dr. Edward F. Scanlon
Performing Organization:	Evanston Hospital
City and State:	Evanston, IL

Contract Number: NO1-CB-53917
Starting Date: 6/30/75 Expiration Date: 6/29/81

Goal: To determine the effects of adding adjuvant chemotherapy and BCG to mastectomy for patients with locally advanced breast cancer.

Approach: Patients who have had Stage II or III breast cancers were admitted to the protocol. Stratification and randomization were carried out along with a balancing for prognostic factors according to the following variables: primary tumor size, number of positive nodes, menopausal status, and unfavorable local signs. The statistical analysis involved sequential treatment assignment and was designed to provide the greatest balance among all groups assigned according to all available prognostic indicators. Using the above method each of the patients in the study was assigned to one of three treatment schedules: (1) oral phenylalanine mustard (L-PAM); (2) 5-Fluorouracil, cyclophosphamide and prednisone (CFP); and (3) CFP plus BCG inoculations. Two of these three treatment schedules are identical with the Mayo Clinic adjuvant study and the data will be cumulative. The statistical method of analysis is also identical with that now being used at the Mayo Clinic. The treatment courses have been given for one year or until there was evidence of recurrence, whichever occurs first. Survival and disease-free intervals will be computed for each treatment group.

Progress: Since July 1975, the study enrolled 194 patients. On July 30, 1979, enrollment was closed. Entry to the L-PAM regimen was discontinued in November of 1977 because of a disproportionately high recurrence rate in that group. Currently there are 13 of 38 patients (38%) with recurrences in the L-PAM group compared to 18 of 78 (18%) in each of the other two groups. Of those patients with recurrences, about 75% had primary tumors greater than 3 cm., and about two-thirds had four or more metastatic axillary nodes. The patients who received polychemotherapy had a higher frequency of nausea, vomiting, diarrhea, alopecia and stomatitis than those who received L-PAM, while L-PAM resulted in more severe thrombocytopenia. Immunologic evaluation was done initially and every three months for the first two and a half years of the study. However, phytohemagglutination analysis and skin tests did not reveal any significant trends among the treatment groups.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $50,000

CONTRACT RESEARCH SUMMARY

Title: Surgical Adjuvant Chemotherapy in Patients with Breast Cancer

Principal Investigator: Dr. David L. Ahmann
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: NO1-CB-33899
Starting Date: 6/30/73 Expiration Date: 6/29/81

Goal: To assess the effects of surgical adjuvant chemotherapy with or without radiation therapy in patients with operable but prognostically unfavorable breast cancer.

Approach: Patients with unfavorable breast cancer (node + or unfavorable signs) were assigned to treatment programs involving mastectomy followed by adjuvant chemotherapy with or without radiation therapy. For the premenopausal group of patients two treatment arms were completed, cyclophosphamide, fluorouracil and prednisone (CFP) with or without radiation therapy. Treatment programs were chosen by random selection. For the postmenopausal group of patients three treatment programs remained open and again chosen by random selection, phenylalanine mustard (L-PAM) versus CFP with or without radiation therapy. Adjuvant chemotherapy was initiated up to six weeks in the postoperative period and given concomitantly with radiation therapy when this modality was utilized. Chemotherapy was continued for a total of ten cycles or upon the appearance of recurrent disease, whichever should occur first.

Progress: Three hundred and one patients were entered on clinical trial and of these 295 are considered evaluable. Recurrence rates for 1000 weeks of observation were 4.6 for L-PAM, 2.9 for CFP and 2.4 for CFP plus radiation therapy. Recurrence rates to date are 46 percent on L-PAM, 32 percent on CFP and 28 percent on CFP plus radiation therapy. In the premenopausal group of patients, CFP with or without radiation therapy is statistically superior to L-PAM, supporting the previous decision to drop L-PAM for the premenopausal group of patients. The differences among recurrence rates for the entire group is almost totally accounted for in the premenopausal group of patients. Radiation therapy added to polychemotherapy does not detract from the program and the suggestion exists that it enhances the treatment program. Myelosuppression occurred in most patients and was equally distributed among the various groups. Minority of the premenopausal patients had significant menstrual disturbances, suggesting that kinetic factors are the major influences on disease-free status and survival. Current analysis of the role that radiation therapy plays in this group of patients suggests that patients with medial quadrant lesions do better with radiation therapy added to the chemotherapy program but statistically this is not significant. No other factors have been identified which would allow one to select a subset of patients who would benefit with the addition of radiation therapy.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds $40,000

CONTRACT RESEARCH SUMMARY

Title: Occult Breast Cancer Metastases in Axillary Lymph Nodes

Principal Investigator: Dr. Edward J. Wilkinson
Performing Organization: The Medical College of Wisconsin
City and State: Milwaukee, WI

Contract Number: NO1-CB-74110
Starting Date: 7/1/77 Expiration Date: 3/31/80

Goal: To determine if the presence of occult metastases in axillary lymph nodes previously diagnosed as negative correlates with the clinical recurrence of breast cancer.

Approach: The investigators have comprehensively restudied the lymph nodes removed from 525 patients at the time of mastectomy for primary breast cancer. In all cases the initial histological examination of the nodes was reported as demonstrating no evidence of metastases. A pilot study on a subset utilized 6 micron serial sectioning of node material and the best spacing between sequential subsets of these slides has been determined by reevaluation of the subsets. Five years or more follow-up information is available on all patients. The study considers the following factors in relation to their risk for clinical recurrence: primary tumor type, grade and size, breast lymphatic or blood vessel invasion, number of nodes removed, histologic character of the nodes, number of positive nodes, volume of tumor in the nodes, age, menstrual history, reproductive history, height, weight, and survival information.

Progress: Clinical follow-up data have been collected from the five hospitals involved in the study. Completed follow-up data have been obtained on all 525 patients with 5 to 10 or more years follow-up. Resectioning of lymph nodes and reexamination have been completed on all cases. The evaluation entailed sectioning of 121,369 slides. Eighty-nine cases have demonstrated occult lymph node metastasis.

The original slides were examined in all cases before acceptance into the study. A total of 677 cases were examined and 525 of these were accepted. Of these cases, occult lymph node metastasis was found on reexamination of the original slides in 18 of the 677 cases of 2.7% of cases initially interpreted as negative.

The significance of occult lymph node metastasis relevant to survival is presently under study. Preliminary analyses of the data indicate no significant difference between those patients showing occult metastases and those whose lymph nodes are negative upon detailed reexamination.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Suppression of Endocrine Function by Systemic Agents for Breast Carcinoma Therapy

Principal Investigator: Dr. Richard J. Santen
Performing Organization: The Pennsylvania State University
City and State: The Milton S. Hershey Medical Center
Hershey, PA

Contract Number: NO1-CB-53851
Starting Date: 5/15/75
Expiration Date: 5/14/81

Goal: To produce suppression of endocrine function with aminoglutethimide (AG), to establish the mechanism of steroid inhibition, and to compare the effects of surgical adrenalectomy, antiestrogen therapy, and aminoglutethimide in human breast carcinoma.

Approach: Female patients with inoperable, recurrent or metastatic breast carcinoma and whose tumors are either estrogen receptor positive or unknown are selected for study. Women are randomized into two separate therapeutic trials: a comparison of medical adrenalectomy (AG + hydrocortisone) vs. surgical adrenalectomy, and of AG vs. the antiestrogen, tamoxifen. Extensive endocrine studies evaluate the effects of AG on extra-adrenal estrogen production, androgen, progestin, and prolactin secretion, and steroid metabolism.

Progress: One hundred and thirty-seven women with metastatic breast carcinoma have been entered into protocols utilizing surgical adrenalectomy, medical therapy with aminoglutethimide (AG) and tamoxifen. Similar response rates to surgical adrenalectomy and AG are being observed in randomized patients. Comparison of the levels of androstenedione, testosterone and dihydrotestosterone in women after surgical adrenalectomy or during AG treatment revealed striking differences. Surgical adrenalectomy markedly lowered the secretion of these androgens whereas AG did not inhibit testosterone or dihydrotestosterone and lowered androstenedione by only 50%. Preservation of androgen production with inhibition of estrogens could be an important mechanism of action of AG in inducing tumor regression. Four women treated initially with surgical adrenalectomy experienced additional estrogen suppression when AG was added. In one, partial objective regression of pulmonary nodules resulted. No patients (0/11) crossed over from AG to tamoxifen have achieved a further objective regression. Systemic studies of adrenal reserve immediately after stopping AG revealed immediate normalization of basal cortisol levels and return of stress responses within 72 hr. Consequently, patients receiving AG do not experience episodes of acute adrenal insufficiency during stress as observed following surgical adrenalectomy. Overall, these studies suggest that medical adrenalectomy with AG and hydrocortisone is an appropriate alternative to surgical adrenalectomy.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $120,900

CONTRACT RESEARCH SUMMARY

Title: Investigation of Steroid Sulfation and Estrogen Binding in Human Breast Cancer

Principal Investigator: Dr. Thomas L. Dao
Performing Organization: Roswell Park Memorial Institute
City and State: Buffalo, NY

Contract Number: NO1-CB-43900
Starting Date: 2/1/74 Expiration Date: 1/31/81

Goal: To determine the relationship of steroid sulfotransferase activity to estrogen receptor protein levels, to pathologic staging, and to risk of relapse.

Approach: Steroid sulfating enzyme activity and estrogen receptor (ER) levels have been assayed in breast tissue obtained from approximately 300 patients in three institutions. The evidence will be evaluated for or against correlation of the steroid sulfotransferase activity with risk for tumor metastasizing potential and with histopathologic parameters. The predictive potentials of ER and sulfation activity will be compared.

Progress: A total of 266 patients from three cooperating institutions was entered into the study between March 1974 and June 1976, inclusive. The average length of follow-up is now approximately 48 months. The data have been analyzed according to the following categories: DHEAS versus other prognostic indicators; E_2S versus other prognostic indicators; ER versus other prognostic indicators; and the recurrence rates for categories of prognostic indicators. The prognostic indicators considered were: age at diagnosis, pathological size of the tumor, histologically positive axillary nodes, and menopausal status. This analysis revealed that DHEAS and E_2S are highly correlated with each other but appear to have little or no correlation with other prognostic indicators for recurrence; that the three hospitals have different kinds of patients when these prognostic indicators are considered; and that the ratio of DHEAS to E_2S appears to be a prognostic indicator for recurrence in this set of patient data.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $10,053

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II Carcinoma of the Breast

Principal Investigator: Dr. Donald L. Morton
Performing Organization: University of California
City and State: Los Angeles, CA

Contract Number: NO1-CB-43917
Starting Date: 6/17/74 Expiration Date: 6/16/81

Goal: To evaluate in patients with Stage II carcinoma of the breast the effectiveness of post-operative adjuvant chemotherapy and two forms of adjuvant chemo-immunotherapy.

Approach: Patients with positive axillary lymph nodes are randomly assigned to one of the following treatment schedules: (1) cyclophosphamide, methotrexate, and 5-fluorouracil (CMF); (2) CMF plus BCG; or (3) CMF plus BCG plus tumor cell vaccine (TCV). The tumor cell vaccine consists of irradiated allogeneic breast carcinoma cells grown in tissue culture. Chemotherapy is administered in 12 cycles for 64 weeks. Immunotherapy is given concurrently and for one year following chemotherapy. BCG is administered by Tine technique; tumor cell vaccine is administered intradermally. Treatment failure consists of either metastases or development of carcinoma in the contralateral breast. The patients are evaluated before treatment and at intervals throughout the study.

Progress: One hundred thirty-one patients have been entered into the protocol since June, 1974. The longest follow-up is 68 months. The recurrence rate for the CMF group is 5/37 (13.5%) at a 33 month mean follow-up. This group is being carefully studied to determine if prognostic factors are the same as in the CMF-BCG and CMF-BCG-TCV group. The recurrence rate for the CMF + BCG group is 22/53 (42%) and for the CMF + BCG + TCV group, 15/41 (37%) at a 49 month mean follow-up. These figures are all significantly better than the recurrence rate for untreated historical controls. There is no significant difference for recurrence and survival between the pre- and postmenopausal groups.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $30,000

CONTRACT RESEARCH SUMMARY

Title: Prediction of Hormone Dependency in Human Breast Cancer

Principal Investigator:	Dr. Elwood V. Jensen
Performing Organization:	University of Chicago
City and State:	Chicago, IL

Contract Number:	NO1-CB-43969		
Starting Date:	6/16/66	Expiration Date:	6/15/81

Goal: To develop and improve techniques that will increase the predictability of response of human breast cancers to endocrine therapy.

Approach: Since 1966, Dr. Jensen has been studying estrogen receptors (ER) in human breast cancer tissues and the relationship between ER content and response to endocrine manipulative treatments. Now that the predictive value of ER measurements has been established, attention is being directed toward development of simple, inexpensive assay procedures for ER in breast cancers and evaluation of the ability of an ER assay on the primary tumor, carried out at the time of mastectomy, to predict subsequent response to endocrine therapy.

Progress: In the attempt to develop a simple immunoradiometric assay for estrophilin in breast cancers, monoclonal antibodies to estrogen receptor protein have been prepared by hybridizing splenic lymphocytes from a Lewis rat, immunized with purified ER from calf uterine nuclei, with three different mouse myeloma cell lines and cloning the resulting hybridomas. Fifty-six monoclonal lines of antiestrophilin-secreting hybridoma cell lines have been obtained, producing either IgG or IgM, but these antibodies, like those in the serum of the parent Lewis rat, react only with ER from calf tissues. Similar immunization of an ACI rat gave rise to antibodies that cross-react with ER of human breast cancer; fusion and cloning of the resulting hybridomas to furnish cross-reacting monoclonal antibody is in progress. A Lewis rat has been immunized with purified ER of MCF-7 cell cytosol to give a good titer of anti-human estrophilin antibody; fusion of splenic lymphocytes from this rat has been carried out successfully and three monoclonal lines of anti-ER-producing hybridoma cells have now been obtained. As an alternative approach to receptor assay, a simple rapid procedure has been developed using controlled pore glass beads, which can provide an estimate both of unoccupied receptor in the tumor cytosol and occupied receptor sites in the nucleus. Tabulation of clinical and receptor data for all patients from the University of Chicago hospitals is now complete and correlations are being made. Tabulation of data for patients from Michael Reese hospital is underway. For mastectomy patients, 15 of 20 women with receptor-rich primary tumors showed objective remission to endocrine therapy (8/10 ablative, 7/10 additive), in contrast to 1 remission of 25 patients with receptor-poor primary tumors.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $66,400

CONTRACT RESEARCH SUMMARY

Title: Effect of Chemotherapy-Induced Endocrine Alterations on Stage II Breast Cancer

Principal Investigator: Dr. Tapas K. Das Gupta
Performing Organization: University of Illinois Medical Center
City and State: Chicago, IL

Contract Number: NO1-CB-84221
Starting Date: 7/1/78 Expiration Date: 6/30/81

Goal: To determine if the prolongation of the clinically disease free interval produced by adjuvant chemotherapy is a result of 1) direct anti-cancer actions, 2) indirect action via endocrine organ changes, 3) combination of 1 and 2.

Approach: In the clinical study, preminopausal women with operable Stage II breast cancer will be registered to obtain preoperative hormone profiles, If one or more axillary nodes contain histologically proven metastases, the patient enters the adjuvant chemotherapy program. Estrogen and progesterone receptor assays are performed. The study patients receive 12 courses of cyclophosphamide, methotrexate, and fluorouracil. Blood hormone levels are drawn during the first 2 wks of each 28 day cycle of chemotherapy for 3 consecutive cycles and every 3 months thereafter during CMF administration. Animal studies will use adult female BUF/N rats and NMU induced mammary tumors. Effects of CMF on tumor, endocrine organs and fecundity will be studied.

Progress: To date one of the 24 evaluable patients receiving CMF chemotherapy has failed. Of 24 tumor cytosols assayed for ER, 17 have been assayed for PGR. The receptor distribution in these cytosols: 5/17 ER+PGR+; 7/17 ER+PGR-; 0/17 ER-PGR+; 5/17 ER-PGR-. Of 15 patients receiving at least 6 courses of CMF, 10 have become amenorrheic. Plasma hormone levels show an upward shift of the LH and FSH values of patients who exhibit amenorrhea. TSH levels also show a trend toward increase in these patients.

In animal studies the carcinogen, 1-methyl-1-nitrosourea, has proven superior to DMBA. Tumor incidence in BUF/N rats is >90%, average latency - 90 days. Growth is linear, appearance is uniform. All lesions are ER positive and respond to ovariectomy. A significant incidence of PGR has been noted. The incidence of cytosol receptor for androgen and glucocorticoid is <20%. Tumor incidence is negligible in BUF/N rats more than 10 months old with atrophic vaginal smear patterns. We have begun administering Cytoxan to control (cycling) and tumor bearing (cycling) BUF/N rats. Cytoxan disrupts vaginal cyclicity. We are evaluating its effect on steroid levels and tumor growth.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $94,140

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator: Dr. John M. Yuhas
Performing Organization: University of New Mexico School of Medicine
City and State: Albuquerque, NM

Contract Number: NO1-CB-74203
Starting Date: 8/1/77 Expiration Date: 7/31/80

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: This project consists of 4 phases: growth of rat breast cancer lines as multicellular tumor spheroids; comparison of the sensitivity of these MTS to 6 drugs (5-FU, MTX, CYC, ADR, VCR and L-PAM) with the sensitivity of the respective tumors growing in vivo; growth of human breast cancers, derived at surgery or biopsy, as MTS; and then comparison of the responses observed in the clinic with those predicted by the MTS grown from the individual patients breast cancer.

Progress: The first two phases of the project have been completed and can be summarized as follows: breast cancer lines, in general, can be grown as MTS unless they were originally obtained from pleural effusions; the growth rate of the MTS is proportional to the growth rate in vivo; and, for at least 4 of the drugs (CYC, ADR, VCR AND L-PAM) the sensitivity of the MTS is predictive of the sensitivity of the tumor growing in vivo. Our progress in growing MTS directly from patients has been limited for a number of reasons: the small sample size of biopsies, which accounts for most of our patient material; our limited success in obtaining samples, however small; and the inherent difficulty of growing tumors directly from the clinic. While our sample availability has improved within the last reporting period (3 new samples), we have not yet devised a way to consistently produce MTS fast enough and in sufficient quantity to satisfy our needs. The problem has, in part, been identified, in that we have been able to demonstrate that the addition of reproductively dead cells to a culture which would produce MTS effectively drastically reduces the yield of MTS. Attempts are being made to purify the reproductively intact cells to overcome this problem.

A second problem identified, which would affect all predictive systems, is that the MTS are predictive of the initial response of the tumor in vivo, but not of the quantitative or qualitative aspects of the drug resistance evolution. We are presently attempting to determine whether in vitro simulation of repeated treatments can predict the evolution of resistance, and how it might be countermanded.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator:	Dr. Russell Hilf
Performing Organization:	University of Rochester School of Medicine and Dentistry
City and State:	Rochester, NY
Contract Number: NO1-CB-74204	
Starting Date: 7/15/77	Expiration Date: 7/14/81

Goal: To develop a reliable prognostic test that will predict the efficacy of chemotherapeutic agents in the treatment of individual breast cancer patients.

Approach: This project performs a battery of selected enzymes in primary and metastatic breast cancer specimens and develops mathematical models based on the biochemical profile. The enzymes include lactate dehydrogenase, pyruvate kinase, glucosephosphate isomerase, isocitrate dehydrogenase, phosphoglucomutase and glucose 6-phosphate dehydrogenase. DNA and protein levels are measured, and hormone receptor assays and histopathologic examinations are performed. Data concerning the patients' biological and oncological histories are abstracted, evaluated and stored. Analyses of the combined data will test the predictive accuracy of the mathematical model constructed from the biochemical profile.

Progress: More than 300 patients have been entered based on the criteria of eligibility, and case review continues on patients whose tumors were obtained during the 7 years prior to initiation of this contract. The logistic regression model using LDH vs. PK x ICD x GPI has classified correctly 65/78 (83%) advanced disease patients for response or no response to chemotherapy regimens. The data set is continually increased in number and subjected to exploratory investigations. We have identified 86 patients who received adjuvant therapy; 42 have recurrence of disease. Preliminary results from 32 patients failing adjuvant treatment (recurrence during therapy or within 12 months after therapy ceased) showed that their tumors had low enzyme profiles, resembling those seen in lesions from advanced disease patients classified as non-responders. This finding suggests a potential application of enzyme data to the adjuvant setting. More than 150 patients with no postmastectomy therapy have been identified and we are evaluating the biochemical properties of their lesions along with several clinical parameters as they relate to recurrence of disease. Analysis of ER status as it relates to response is being performed; our data show that in contrast to the enzyme profile, ER offers no useful prognostic value for response to chemotherapy or for failure of adjuvant therapy.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $10,000

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator: Dr. Daniel D. Von Hoff
Performing Organization: University of Texas Health Science Center at San Antonio
City and State: San Antonio, TX

Contract Number: NO1-CB-74205
Starting Date: 8/1/77
Expiration Date: 7/31/80

Goal: To develop a reliable test system for predicting effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: In vitro effect of drug-containing plasma and of direct drug addition in pharmacologic concentrations on the thymidine labeling index (LI) of tumor cells is assayed over time. These effects are correlated with in vivo effects on tumor size in 13762 tumor after one drug dose.

Progress: Recent months have been devoted to duplicating previously run experiments using an improved (hybrid) tissue culture method. One milliliter of cells suspended in double-enriched RPMI 1640 is layered over agar base plates. Labeling indices (LI) are done at appropriate time points. This system has the advantages of maintaining higher viabilities and higher LI for a longer period of time. Results are theoretically obtainable in 8-9 days and there is no problem separating the cells from the agar. We have compared 24 hr labeling to 1 hr "flash" labeling indices and have found 24 hr labeling to be significantly higher. At 144 hr, the average viability of 8 control cultures was 35% (trypan blue dye exclusion). The average 1 hr LI of 4 control cultures was 8% whereas the average 24 hr LI for the same time period was 25%.

Preliminary analyses indicate good results obtained for adriamycin and methotrexate (both negative correlations), 5-fluorouracil, cytoxan, and the combinations of cytoxan-vincristine, and cytoxan-adriamycin (positive correlations). Two experiments, one each for vincristine and L-PAM (melphalan) did not correlate. In the vincristine experiments, the in vitro predicted non-response, while the in vivo response to treatment was 50% reduction. In the L-PAM experiment there was an in vitro prediction for response (treated plasma LI less than 50% control plasma at 120, 144, 168 hours in culture) but no in vivo response.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Biochemical Mechanism of Endocrine Induced Breast Cancer Regression

Principal Investigator: Dr. William L. McGuire
Performing Organization: University of Texas Health Science Center
City and State: San Antonio, TX

Contract Number: NO1-CB-23862
Starting Date: 6/1/72
Expiration Date: 5/31/81

Goal: To obtain new information of the hormonal control of breast tumor growth and to identify those breast cancer patients whose disease will respond to hormonal manipulation.

Approach: To provide correlations of estrogen and progesterone receptor measurements with prognosis at the time of mastectomy and response to endocrine therapies. To evaluate histochemical and immunological methods of measuring steroid receptors.

Progress: We have previously reported in a small pilot series that patients with estrogen receptor negative (ER-) tumors recur early regardless of tumor size, location, axillary nodal status or age. These data have now been extended to show that survival is also worse in patients with ER- tumors. We are obtaining follow-up data regarding recurrence and survival and are reviewing the histopathology in several hundred additional patients in whom we have previously assayed their primary tumor for receptor content. In this large series of patients none has received systemic chemotherapy or endocrine therapy until recurrence and they can serve as the reference point for current adjuvant protocols employing ER stratification where a surgery alone treatment arm is lacking. We originally hypothesized that the measurement of progesterone receptor (PgR) in addition to ER would improve the ability to select patients with advanced breast cancer for endocrine therapy. Since the presence of PgR is correlated with absolute amount of ER which in turn correlates with clinical response, it is important to know if PgR provides more information than quantitative ER alone. We are now critically examining this question since we have more than 100 patients with quantitative levels of both ER and PgR and data concerning response to endocrine therapy.

In a large series of experiments designed to test whether antibodies to estradiol can be used to identify ER containing breast tumors, our findings are unequivocal. We are now trying to evaluate the alternative approach of using fluorescein coupled estradiol to directly detect ER. Concerning antibody to ER, the monoclonal hybridoma technique is promising. The major effort is to obtain suitable ER antigen from MCF-7 cells and we are exploring additional techniques to increase cell and/or receptor yield.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
FY 80 Funds: $119,000

CANCER DIAGNOSIS RESEARCH PROGRAM

The Cancer Diagnosis Research Program is an extramural program consisting of both contracts and grants administered by the staff of the Diagnosis Branch in the Collaborative Research Program of the Division of Cancer Biology and Diagnosis, NCI. The program this year includes immunodiagnosis contracts, which formerly were administered by the Immunology Program, DCBD, and all of the grants concerning cancer detection and diagnosis which formerly were administered by the Division of Cancer Research Resources and Centers.

Some research in cancer detection and diagnosis is not included in the Cancer Diagnosis Research Program; instead, it is included as integral parts of projects supported by the Division of Cancer Treatment and by the Division of Cancer Cause and Prevention. In those programs major emphasis is on either treatment or cause and prevention and the detection or diagnosis segments represent portions of the larger program.

The present compilation includes a summary of each contract and grant currently in the Cancer Diagnosis Research Program plus those recently completed. For completeness a brief summary of past accomplishments is included.

PAST ACCOMPLISHMENTS

The Cancer Diagnosis Research Program has been in existence as such since 1973 although several contracts in cancer diagnosis began before that time. Most of the work completed at this time has been accomplished by some 45 contracts.

An early study in lung cancer screening led to the experimental design used in the current studies at three institutions. Studies on pancreatic cancer diagnosis at three institutions have demonstrated the extreme difficulty of early detection and have provided leads toward further diagnostic improvements. Currently, ultrasound, CT scanning and retrograde pancreaticoduodenography can identify pancreatic cancers but not at a sufficiently early (localized) stage to improve treatment results. Three contracts have completed basic studies to test possible differences in nuclear magnetic resonance (NMR) signals in normal and tumor tissue. By the methods employed it was not clear that NMR would distinguish between normal and tumor tissue, however, the studies helped to define the problems requiring solution if NMR imaging is to be useful in cancer detection. Two studies have attacked the difficult problem of optimal frequency of screening; several reports concerning those results have been published. Two studies have investigated chemical and immunological methods to more sensitively detect human blood in feces but no completely satisfactory procedure has been found. Two studies have been completed which dealt with improvement of x-ray imaging by removing scatter. One study, completed in 1976, dealt with proton beam absorption in normal and tumor tissue specimens as a preliminary to other proton beam imaging studies. The

proton beam absorption was shown to be sensitive to slight differences in tissue composition and the results suggested possible usefulness of proton beam imaging for tumor detection. Another study, now supported as a grant (since 1979) after five years as a contract, used protons as well as helium and carbon ions to study imaging in both tissues and human subjects. The extremely promising results obtained are being followed up in the present grant. Several contracts supported the development and cataloging of algorithms useful for x-ray and radionuclide imaging. Those algorithms are made available to other workers for further studies. A data base was also developed for testing and studying x-ray algorithms. Development of an important new design for a x-ray CT body scanner was supported by the contract research program. Preliminary work on development of an x-ray absorbing electrophoretic imaging display system was carried out in a two year contract now terminated. Possible further work to develop such an imaging display system may be carried out in new initiatives. A standard protocol was recently completed which provides guidance for studies to compare and evaluate imaging techniques used in cancer diagnosis.

Studies have been carried out to develop new methods for isolation and characterization of human peripheral blood lymphocytes. Seven recently completed contracts have produced a variety of interesting results on diagnostic aspects of tumor-associated antigens; these include 6 contracts involving cervical and ovarian tumor associated antigens, leukemia associated antigens, and various other circulating antigens. Development of radionuclide labelled immunoglobulins for external localization of tumors was successful in one recently completed contract. Two completed contracts studied development of immunologic markers which would be used in cytology automation. Hormone markers for detection and diagnosis of cancer were extensively studied in one five year contract. Another contract investigated the suitability of a macrophage electrophoretic mobility test to detect and monitor malignant disease. The effect of suppressor monocytes on cellular immune function and the possible presence of any useful markers was investigated in one contract study.

In contrast to the 45 completed contracts only 5 grants have been completed since the diagnosis grants were added to the Cancer Diagnosis Branch Program in 1978. Those studies included work on circulating thyroglobulin levels in thyroid carcinoma; an in depth study of high risk factors in breast cancer; a study of methods for immunodiagnosis of cancer with human chorionic gonadotropin (hCG) detection; a study to develop an improved clinical test for serum acid phosphatase to aid in diagnosis of prostate cancer; and a group of experiments to investigate development of coded-aperture systems for improved imaging with radionuclides.

Results obtained in the completed contracts and grants briefly mentioned above have been reported in numerous journal articles. A substantial number of articles have also been published in appropriate journals in relation to various current contracts and grants supported by the Cancer Diagnosis Research Program.

DESCRIPTION OF THE CANCER DIAGNOSIS RESEARCH PROGRAM

This program emphasizes research in early detection, diagnosis and monitoring of cancer as well as the application of this knowledge to the appropriate populations for clinical evaluation. Projects in these areas are concerned with improvement of existing techniques as well as development of new tests and procedures. Research in detection and diagnosis attempts to recognize and localize cancer in the earliest possible stage. Such recognition should improve the chances for control of the disease and increase the duration and quality of life. The program seeks to establish modalities for early recognition in general as well as an improved definition of early lesions in specific body sites. Research in cancer diagnosis also includes the development of knowledge, methods and techniques to establish the presence, location and extent of cancer and precancerous conditions and prognostic indications. In addition, it seeks to evaluate the clinical efficacy of these approaches. It should be recognized that, in the earliest stages of development, research may not be site oriented. Also in such early stages, research efforts may be involved with animal model systems as well as with human disease.

Formerly, when the Program was composed entirely of contracts, it was described as being comprised of five specific areas of involvement: General Cancer Diagnosis, Diagnostic Radiology, Cytology Automation, Breast Cancer Diagnosis and Immunodiagnosis. The first three areas were the province of the Diagnosis Branch, DCBD, while Breast Cancer Diagnosis was directed by the Breast Cancer Task Force of DCBD, and the Immunodiagnosis Contract Program was operated by the Immunology Program, DCBD.

After the reorganization at NCI, during 1978, when the diagnosis grants were added to the Diagnosis Program it has become convenient to classify both contracts and grants in a more unified system. The present system has eight disciplinary categories: Biochemistry, Immunodiagnosis, Cytology, Pathology, Radiological Imaging, Non-Radiological Imaging, Nuclear Medicine and Multiple Disciplines. The distribution of contracts and grants currently in the Cancer Diagnosis Program into the various categories is summarized in the following table. Each category is discussed in the sections following the table.

CANCER DIAGNOSIS RESEARCH PROGRAM

Number	Category	Grants		Contracts	
		Number	FY-80 Funding	Number	FY-80 Funding
1	Biochemistry	18	1,366,168	2	0
2	Immunodiagnosis	14	1,500,335	37	1,320,013
3	Cytology	12	1,252,813	9	1,641,708
4	Pathology	4	304,486	0	0
5	Radiological Imaging	14	1,201,249	3	421,306
6	Non-Radiological Imaging	11	1,010,562	2	0
7	Nuclear Medicine	17	1,202,060	1	0
8	Multiple Disciplines	1	73,116	14	3,282,590
	TOTALS	91	$7,910,789	68	$6,665,617
	GRAND TOTALS			159	$14,576,406

1. BIOCHEMISTRY

Biochemical methods to improve diagnosis and detection of cancer involve the study of a variety of substances such as hormones, enzymes, proteins and metabolic products in the circulation and in other biological fluids, as well as the study of surface characteristics of tumor cells and chemical characterization of tumor cells. Measurement and mode of action of the multiple forms of human growth hormone are being studied on grant CA-14025. A variety of enzymes are being studied for their correlation with different cancers. Histaminase and an isozyme of alkaline phosphatase are being investigated as general cancer markers by grant CA-26652 and contract CB-74173 respectively. 5'-Nucleotide phosphodiesterase has demonstrated ability to detect liver metastases in patients with breast cancer under contract CB-64029. A profile of enzyme patterns is being studied to help characterize and classify human lung cancer under grant CA-25016 and enzymes in plasma and cells are being monitored during the treatment of patients with lymphoma to provide guidance for the therapy under grant CA-22065. Serum, urine and CSF ribonucleases in healthy subjects, and cancer patients is the subject of grant CA-19606. Protein analysis of pancreatic secretions is being studied as an aid to diagnosis of pancreatic cancer by grant CA-14380. Chemistry of serum lipoproteins is being studied to determine whether qualitative as well as quantitative differences occur between mammary carcinoma patients, normal individuals with a family history of cancer and those without family history of cancer in grant CA-19634.

A "myasthenic substance" found in small cell lung cancer is being studied as a possible aid in detection of lung cancer by grant CA-22885. Serum levels of a heteroglycan fraction which have been found to be markedly elevated in tumor-bearing animal models, are being evaluated for diagnostic sensitivity and reliability in cancer by grant CA-21958. Studies are in progress on biochemical detection and identification of the entire range of dihydroxyphenylalanine (DOPA) metabolites and other reducing compounds in urine of malignant melanoma patients. Patterns of these metabolites were found to differ greatly from normal DOPA metabolism in grant CA-20423. The usefulness of guanosine monophosphate (cyclic GMP) and adenosine monophosphate (cyclic AMP) assays is being evaluated as a potential measure of cellular proliferation and also, detection, diagnosis and treatment of estrogen-dependent neoplasms in human females by grant CA-25357. Urinary nucleoside breakdown products of tRNA are being measured in cancer patients in grant CA-25210 to explore molecular mechanisms responsible for increased excretion levels in cancer patients. In grant CA-14185 identification of human RNA metabolites excreted in urine of cancer patients and normal subjects is being studied. In studies on human chondrosarcomas, structural changes which occur in components of the intracellular matrix are being investigated as indicators of the degree of malignancy by grant CA-23945. Estrogen receptors are being studied in breast cancer and also the patient's response to endocrine manipulation by grant CA-23848, in an attempt to develop a direct method of determining fluorescent hormone uptake in hormonally dependent cancers as well as in normal target tissues such as endometrium and mammary gland.

Differentiation of lymphocytes by the enzyme marker, terminal deoxynucleotidyl transferase (TdT) is also being investigated on grant CA-22509 by determination of the relationship of presence or absence of the enzyme to particular cell sets and to tumors representing those cell sets. The cupric ion bound to ceruloplasmin is being determined by electron paramagnetic resonance measurements made in both cancer patients and normal subjects in grant CA-14335. A major effort is being made under grant CA-23922 to obtain purified epithelial cells from normal, benign, and malignant human mammary glands by developing methodology for their disaggregation, purification, and characterization using biochemical and immunochemical techniques.

2. IMMUNODIAGNOSIS

The portion of the Diagnosis Program classified as Immunodiagnosis can be subdivided into projects dealing with circulating tumor antigens or markers, projects dealing with antibodies to tumors (including immune complexes) and studies of cell mediated immunity. The carcinoembryonic antigen is the most widely utilized circulating tumor marker and there are two contracts dealing with the prognostic and monitoring value of CEA following therapy of colorectal and lung cancer: CB-23854 and CB-33858. Additionally there are studies on the heterogeneity of CEA, grant CA-24376, and comparison of new gastrointestinal cancer antigens with CEA, contract CB-84259, grants CA-04486 and CA-26246.

Several projects are investigating the relationship between hormone levels and cancer; calcitonin, and beta-lipotropin are subjects for two grants, CA-22137, and CA-23382. Two studies of serum calcitonin as a screen for family members of patients with medullary thyroid cancer are supported by grant CA-22595 and contract CB-63994. Two studies of enzymes as markers for cancer deal with tyrosinase in patients with melanoma, grant CA-25381, and UDP-galactosyltransferase in patients with ovarian and breast cancer, contract CB-84260. Proteins or their degradation products have been detected in body fluids and have demonstrated correlation with the presence of cancer. Studies of some of these are in the developmental phase, grant CA-24645 and contract CB-84308, while the fetal sulfoglycoprotein antigen in gastric juice is being studied in a large detection trial for stomach cancer by contract CB-64070. New tumor associated antigen studies are under way for many organ sites: prostate, CA-23990 and CB-74169; breast CA-20286 and CB-74162; cervix, CB-84267; ovary, CB-64013 and CB-64028; leukemia and lymphoma, CB-84261; melanoma, CB-74127; mesothelioma, CA-27081 and hepatoma in rodents, CB-74167. HLA antigens present on 45 human tissue culture cell lines derived from tumors will be determined and related to HLA phenotype of donor lymphocytes under contract CB-84255. Studies of nuclear antigens associated with normal and leukemic human blood cells to determine their usefulness as markers in hematopoiesis are being supported by grant CA-26948. In addition there are contract studies to investigate the relationship between microbial products and tumor antigens, CB-74122, and the production of a lymphotoxin by lymphoblastic cells in tissue culture, contract CB-84263.

Circulating antibodies to tumor antigens are studied in patients with melanoma, contract CB-74120, while the presence of circulating immune complexes are being quantitated and analyzed in a number of cancers including colon, breast and melanoma by contract CB-84262. Antibodies are also serially measured in mice while developing induced tumors, contract CB-74134. Xenoantibodies to various tumor markers are being used to classify malignant tissues by contract CB-84257 using immunohistochemical techniques. Methods of labeling antibodies by radionuclides for tumor radioimmunodetection and localization are being studied under two grants: CA-25585 for anti-CEA and CA-28462 for anti-HSA. Various parameters of cell mediated immunity are being studied under contracts to define the functional activity of monocytes, CB-74121; the cryopreservation of lymphocytes and monocytes with cytotoxic activity, CB-74131 and CB-74164. The definition of subpopulations of leukocytes using separation techniques depending on specific antibodies or lectins is the subject of three studies: CB-74132, CB-74163 and CB-74129. Studies of natural cytotoxic activity of leukocytes are supported by two contracts, CB-74133 and CB-74166, and the augmentation of cytotoxic activity by another contract, CB-74130. An evaluation of the leukocyte adherence inhibition assay for cancer diagnosis is being performed by contract CB-80316.

In addition to the research grants and contracts there are contracts for the collection and storage of serum samples from patients with cancer and other acute and chronic diseases and during the course of therapy for certain cancers. These contracts, CB-33914, CB-74124, CB-74210, and CB-84258, have been invaluable for the rapid evaluation of a wide variety of serologic tests for cancer that come to the attention of the NCI. A tissue culture bank for cell lines which can be utilized by any investigator for research in cancer immunodiagnosis is maintained under a contract, CB-43854.

3. CYTOLOGY

Diagnostic cytology research projects include the development of automated instrumentation and cell markers that can be used to differentiate normal and atypical cells. Instruments undergoing development and testing are those of high resolution slide based, grant CA-13271 and contract CB-33873, and flow fluorometric types, contracts CB-60311, CB-40300 and CB-33862. Development, construction and testing of an ultrafast optical scanner for microscopic specimens, grant CA-24466, will make possible the automated search for abnormal, transformed or cytochemically marked cells in microscopic preparations. Single cell classification algorithms employing the current state of the art in digital image processing, scene segmentation and image acquisition hardware are being developed and tested on contract YO1-CB-70314. The classification algorithm chosen with human interaction was found to perform at an acceptable error rate.

Flow instruments currently undergoing testing use dual staining and analysis with dual laser beam sorters, contract YO1-CB-60311 and YO1-CB-40300. In addition, a slit-scan using a combined static and flow system is now being tested to determine the system characteristics including rate

and causes of false alarms, contract CB-33862. Quantitative descriptors of normal and abnormal cells include cytochemical and immunological markers which increase the potential sensitivity and specificity of sensor systems.

Chemical synthesis of cytochemical probes, grant CA-24363, with sharp fluorescence emission spectra will enhance multiple staining of cells for flow systems and for cells on slide in static systems. Cellular fluorescent markers under investigation also include studies of fluorescent substrates for demonstration of cAMP phosphodiesterase, adenyl cyclase, and acid phosphatase, grant CA-19552; acridine orange, contract CB-33862; and other DNA protein fluorescent dyes, contracts YO1-CB-40300 and YO1-CB-60311. Cell surface antigens may also be used as markers for abnormal cells. With appropriate fluorescent tagging of these antigens, normal and abnormal cells may be differentiated by automated instruments. Potentially useful markers being studied under contracts are total HSV viral antigens, AG-e, contract CB-74170, antigens extracted from HSV-1 and HSV-2 transformed cells, contract CB-74171, and nucleolar antigen, grant CA-28771.

In order to analyze the performance of automated systems, comparison of instrumental or system classification and manual microscopic classification utilizing standard morphologic criteria has been started under contract CB-74190. In addition, programming of cell recognition algorithms continues with emphasis on scene segmentation techniques in the same contract. Research is in progress, contract CB-74095, to test a module sorter to separate cells based on individual cell parametric measurements. This module will be interfaced with a flow instrument, contract CB-33862, to assure a correlation of individual separated cells with specific quantitative measurements.

Flow cytometry is used in grant CA-27283 to develop an assay for detecting transformed cells (by chemical carcinogens) differentially labeled with fluorescamine. In addition the nature and functional significance of a hyaluronidase sensitive "barrier" surrounding transformed cells is being investigated.

To better understand and treat lymphoproliferative disease, flow microfluorometry analysis of human lymphoid malignancies is under investigation, grant CA-23393, with the hope that malignant subpopulations can be identified by two parameter analysis for size and monoclonal surface immunoglobulin. In addition, it is hoped that the ploidy and cell cycle kinetic parameter of these cells will aid in the diagnosis, classification, scheduling and monitoring of treatment. A combined flow cytometric/cell sorting autoradiographic technique is being developed in grant CA-25348 to monitor acute leukemias of adults and children and to predict relapse and evaluate treatment protocol. Using fluorescence microscopy and flow microfluorimetry, grant CA-27123, merocyanine 540 is employed to study staining reaction in leukemia to characterize the dynamic changes in the staining pattern during the clinical course and to isolate and characterize hematopoietic progenitor cells. The selective

staining of leukemic cells might be a useful adjunct to existing methods of monitoring the course of leukemia and of prognosis of relapse or remission. Leukemic cells are also being studied to determine their proliferation and differentiation in semi-solid matrix culture using cytologic markers, to correlate markers of abnormal growth with clinical alterations, and to test chemotherapeutic agents for their ability to alter patterns of cell differentiation in vivo, grant CA-22942. Study of human leukemic and pre-leukemic blood and bone marrow using the soft agar culture techniques has continued, grant CA-17353, to characterize the status and prognosis of hematoproliferative disorders. Measurements of the quality and quantity of colony formation appear to provide useful indicators of disease status and progression as well as diagnosis and predictions concerning response to therapy of acute non-lymphocytic leukemias and preleukemic states.

In an effort to predict the biological potential of gynecologic neoplastic lesions, a quantitative analysis of nuclear DNA content using Feulgen microspectrophotometry is under way, grant CA-24932. Determination of the nuclear DNA changes may reveal the correlation and association of the changes with regression, persistence and progression of squamous cancer of the uterine cervix and its precursors. Earlier findings of carcinoma-in-situ of lung, intestine, ovary and bladder might be possible by using an immunocytologic technique being evaluated in grant CA-26863, to detect carcinoembryonic antigen on exfoliated cells. An extensive study to test the feasibility of screening for endometrial cancers in asymptomatic women by means of uterine sampling is being done under contract CB-84233.

4. PATHOLOGY

In grant CA-21523, identification and enumeration of all lesions from whole breast using a sub-gross method showed elevated frequencies of atypical lobules and other microscopic lesions in breasts of patients with cancer; it was concluded that almost all lesions are located in the terminal ductular alveolar region and that certain specific lesions have prognostic importance. A system of classifying human pituitary adenomas based on electron microscopy and immunocytochemistry has been developed on grant CA-21905. To expand understanding of tumor behavior in the pyriform sinus and the oral cavity, whole organ sections of patients who undergo surgical excision have been studied, on grant CA-22101, using microscopic appearance to determine size and extent of tumor, relation of tumor spread to the laryngeal framework and specific routes of tumor spread. Malignant lymphomas and leukemias are being studied on grant CA-26422 by a combination of methodologies along with routine morphology to determine the most valuable tests to provide earlier detection and more precise diagnoses.

5. RADIOLOGICAL IMAGING

Several contracts and grants to improve x-ray imaging currently are under study. One contract, CB-74211, seeks to develop large area solid state image receptors for x-ray imaging. A grant, CA-16543, is studying charge

transfer electroradiography. Efforts either to improve x-ray imaging and/or reduce x-ray exposure are subjects of several studies: contrast agents to improve CT scanning studies of the liver, contract CB-84234; development of algorithms for x-ray dose reduction in CT scanning, contract CB-84235; image reconstruction from incomplete projections, grant CA-23818; computer analysis of bone tumor roentgenograms, grant CA-06263; study of optical quality effects on lung tumor detection, grant CA-23816; study of diagnostic accuracy and x-ray image properties, grant CA-24625; and improved diagnostic quality of radiographic imaging for cancer diagnosis, grant CA-24806. Improved imaging of breast tumors is the subject of several grants: CA-22803, computer evaluation of mammographic calcifications; CA-25372, new fluorescent x-ray tubes for mammography; CA-19622, low dose screening techniques for mammography; CA-19787, low dose breast electron radiography; and CA-26327, test phantoms for optimizing mammographic techniques. Other grants related to radiological imaging include CA-15882, construction of a transverse section x-ray camera; CA-23246, recognition of very small tumors in experimental animals; and CA-24822, study of a 3-D tomogram viewer for cancer detection and therapy.

6. NON-RADIOLOGICAL IMAGING

Imaging studies in the Cancer Diagnosis Research Program involving methods other than x-ray and radionuclides include use of proton and heavy ion beams, ultrasound, and thermography. Grant CA-27021, continuing work begun on contract CB-40302, supports a study of the imaging potential of proton, helium, and carbon ion beams; extremely small tissue density differences are detected by ion beams but their lateral resolution is inferior to that of x-ray. Imaging with thermography at microwave and millimeter wavelengths is being studied on grant CA-17642 while grant CA-23142 is concerned with thermography enhanced by microwave and acoustic energy irradiation. Several grants are investigating means to improve breast imaging with ultrasound: CA-19019, ultrasound mammography; CA-23616, new ultrasonic methods for breast cancer detection; CA-24085, quantitative ultrasound imaging of the breast; CA-24257, breast tumor diagnosis by computed ultrasound; CA-25323, ultrasonic computed tomography of breast cancer; and CA-25634, development of an ultrasonic breast tissue phantom. Contrast agents for ultrasound diagnosis are being developed in contract CB-84236 and an ultrasonic probe suitable for use in endoscopes is the goal of contract CB-74136. A scanning acoustic microprobe for cancer diagnosis by external imaging is under development and testing on grant CA-25938. Transpelvic ultrasonic evaluation of the prostate to improve early detection of prostate cancer is being studied on grant CA-27895.

7. NUCLEAR MEDICINE

The major thrust of grants and contracts in the Cancer Diagnosis Research Program which use radionuclides concern tumor-seeking or tumor-delineating agents. Several grants are devoted to production of a variety of tagged tumor-seeking agents: CA-08349, CA-15787, CA-16861, CA-18153, CA-19898, CA-24344, CA-26371, CA-26968, and CA-28561. Grant CA-27252 is carrying

out studies of metabolism of bone scanning radiopharmaceuticals. Contract CB-64011 and grant CA-17742 deal with production of, and localization studies with, labelled immunoglobulins against CEA. Grant CA-22077 is studying zinc radiopharmaceuticals for ocular melanoma detection; grants CA-22578 and CA-24597 deal with imaging melanomas in general. Grant CA-24879 is studying malignant perfusion applied to diagnosis and therapy. Program project grant CA-23417 is concerned with several basic studies to develop an improved nuclear imaging system for tumor detection. Grant CA-28105 involves development of an electronically collimated gamma tomography system for tumor imaging.

8. MULTIPLE DISCIPLINES

A number of contracts and one grant in the Cancer Diagnosis Research Program involve several disciplines and therefore are presented in this last category of multiple disciplines. Five contracts, CB-43910, CB-43980, CB-43981, CB-43982, and CB-43983, have spent approximately five years in a collaborative study to evaluate computerized tomographic x-ray imaging for cranial tumors and compare this with radionuclide imaging. Those studies are now completed and reports are being written for publication. Contract CB-84232 is studying various diagnostic techniques including imaging methods to improve pancreatic cancer diagnosis. Radionuclide markers for tumors on or near endoscopically approachable surfaces and appropriate detectors for them which can be used in an endoscope are being developed on contract CB-64012. Grant CA-25582 is studying fluorescence endoscopy and photoradiation therapy with porphyrin derivatives. Contracts CB-74114 and CB-74212 are developing attachments for the colonoscope to facilitate its easier passage to the cecum. A collaborative study to determine the potential of x-ray imaging and cytological examination of sputum in detection of early lung cancer is being conducted under contracts CB-45007, CB-45037, and CB-53886. A fourth related contract, CB-43868, is concerned with the data management of the collaborative lung study. The longitudinal study, began in 1974, involves 30,000 men and will require several more years of follow-up before definitive results will be available. A large scale longitudinal colon cancer screening study using the Hemoccult test for human blood in the feces is the task of contract CB-53862. Several more years of follow-up on the 45,000 subjects will be required to define the usefulness of the screening procedure in detecting early colon cancer.

LIST OF CURRENT GRANTS

BIOCHEMISTRY

RO1-CA-14025 Multiple Forms of HGH: Measurements and Actions
Willard Vander Laan Scripps Clinic and Research Foundation

RO1-CA-14185 Modified Nucleosides in Cancer and Normal Urines
Girish Chheda Roswell Park Memorial Institute

RO1-CA-14335 EPR Studies on Detection and Treatment of Cancer
John D. Zimbrick University of Kansas Lawrence

RO1-CA-14380 Pancreatic Ductal Versus Duodenal Secretions
Thomas T. White University of Washington

RO1-CA-19606 Serum Urine and CSF Rnases in Health and Disease
Charles A. Dekker University of California Berkeley

RO1-CA-19634 Serum Lipoproteins in Patients with Breast Cancer
Frederick Aladjem University of Southern California

RO1-CA-20423 Studies on the Pathobiological Chemistry of Melanoma
Marsden S. Blois University of California San Francisco

RO1-CA-21958 Cancer Detection by Serum Analysis of Heteroglycans
James D. Morre Purdue University

RO1-CA-22065 Lymphoma: Diagnosis by Blood and Liver Tests
Annemarie Herzfeld New England Deaconess Hospital

RO1-CA-22599 Programs of Normal and Malignant Lymphocytes
Allen Silverstone Sloan-Kettering Institute for Cancer Research

RO1-CA-22885 Lung Cancers and Carcinomatous Neuromyopathies
Koichi Ishikawa University of Southern California

RO1-CA-23848 Steroid Uptake in Hormonally Dependent Cancers
George H. Barrows University of Louisville

RO1-CA-23922 Breast Cancer
Thomas G. Pretlow II University of Alabama in Birmingham

RO1-CA-23945 Assessment of Malignancy in Human Chondrosarcomas
Lawrence C. Rosenberg Montefiore Hospital and Medical Center

RO1-CA-25016 Human Lung Neoplasms: Applications of Enzyme-Pathology
Olga Greengard Mount Sinai School of Medicine

RO1-CA-25210 Origins of Urinary Nucleosides in Tumor Tissue
Ernest Borek AMC Cancer Research Center and Hospital

RO1-CA-25357 Cyclic GMP levels in Gynecologic Neoplasms
Chandralekha Duttagupta Yeshiva University

RO1-CA-26652 Histaminase as a Biochemical Marker for Human Cancer
Chi-Wei Lin Massachusetts General Hospital

IMMUNODIAGNOSIS

PO1-CA-04486 Pathology of the Digestive Tract Mucous Membrane
Norman Zamcheck Boston City Hospital

RO1-CA-20286 Breast Neoplasia Diagnosis with Specific Antibodies
Roberto L. Ceriani Children's Hospital Medical Center Northern California

RO1-CA-22137 Calcitonin as a Marker for Cancer of the Breast
Omega L. Silva Howard University

RO1-CA-22595 Detection of Medullary Thyroid Cancer in Families
Charles E. Jackson Henry Ford Hospital

RO1-CA-23382 Investigation of Human B-Lipotropin
Eckehart Wiedemann University of California Berkeley

R23-CA-23990 Immunodiagnosis of Prostatic Cancer
Ching-Li Lee Roswell Park Memorial Institute

RO1-CA-24376 Immunological Heterogeneity of CEA
James F. Primus University of Kentucky

RO1-CA-24645 Significance of GP48 in Diagnosis of Breast Cancer
Zoltan A. Tokes University of Southern California

RO1-CA-25381 Tyrosinase as a Marker for Human Malignant Melanoma
Kenji Nishioka University of Texas System Cancer Center

RO1-CA-25584 Clinical CEA-Tumor Radioimmunodetection
Frank Deland University of Kentucky

RO1-CA-26246 Assay of Human Tumor or Organ-Associated Antigens
Calvin A. Saravis Boston City Hospital

RO1-CA-26948 Nuclear Antigens as Markers in Hematopoiesis
Robert C. Briggs Vanderbilt University

RO1-CA-27081 Immunodiagnosis of Mesothelioma
Gurmukh Singh University of Pittsburgh

R01-CA-28462 Radiolabeling of Tumor Antibodies
William C. Eckelman George Washington University

CYTOLOGY

R01-CA-13271 Automated Cancer Cell Diagnosis by the TICAS Method
George Wied University of Chicago

R01-CA-17353 Marrow Culture Studies in Human Myeloid Leukemias
Malcolm A. Moore Sloan-Kettering Institute for Cancer Research

R01-CA-19552 New Fluorescent Markers for Cancer Diagnosis
Kwan C. Tsou University of Pennsylvania

R01-CA-22942 Differentiation of Cultured Leukemic Cells
Sandra R. Wolman New York University

R01-CA-23393 Flow Analysis of Human Malignant Lymphoid Cells
Raul C. Braylan University of Florida

R01-CA-24363 Development of Lanthanide Fluorescent Stains
Lidia M. Vallarino Auburn University

R01-CA-24466 Ultrafast Scanner Microscope in Laboratory Automation
Roland V. Shack University of Arizona

R01-CA-24932 Microspectophotometric Nuclear DNA Study of Gynecologic Cancers
Yao S. Fu Case Western Reserve University

R01-CA-25348 Flow Cytometry/Autoradiography Monitoring of Leukemia
Michael Andreeff Sloan-Kettering Institute for Cancer Research

R01-CA-26863 Identification of CEA in Cytology Specimens
Robert R. Pascal St. Luke's-Roosevelt Institute for Health Sciences

R01-CA-27123 Application of Fluorescent Probes to Clinical Cancer
Jay E. Valinsky Rockefeller University

R01-CA-27283 Early Detection of Transformed Cells
Susan P. Hawkes Michigan Molecular Institute

R13-CA-27479 Engineering Foundation Conference on Automated Cytology
Myron R. Melamed United Engineering Trustees

R01-CA-28771 Cytology Automation
Barthel Barlogie University of Texas System Cancer Center - M.D. AndersonHospital and Tumor Institute

PATHOLOGY

RO1-CA-21523 Quantitative Studies of Human Breast Pathology
Sefton R. Wellings University of California Davis

RO1-CA-21905 Pituitary Adenomas: Structure-function relations
Calvin Ezrin Cedars-Sinai Medical Center

RO1-CA-22101 Study of Head and Neck Cancer by Serial Section
John A. Kirchner Yale University

RO1-CA-26422 Clinico-Biologic Correlation in Lymphoma and Leukemia
Henry Rappaport City of Hope National Medical Center

RADIOLOGICAL IMAGING

RO1-CA-06263 Computer Analysis of Tumor Roentgenograms
Gwilym S. Lodwick University of Missouri

RO1-CA-15882 Transverse Section X-Ray Camera
Gordon L. Brownell Massachusetts General Hospital

RO1-CA-16543 Investigation of Charge Transfer Electroradiography
Ivor Brodie SRI International

RO1-CA-19622 Low-Dose Screening Techniques for Mammography
Daniel Kopans Massachusetts General Hospital

RO1-CA-19787 Low Dose Breast Electron Radiography (Ionography)
Gary S. Shaber Thomas Jefferson University

RO1-CA-22803 Computer Evaluation of Mammographic Calcification
Myron Moskowitz University of Cincinnati

RO1-CA-23246 Recognition of Very Small Tumors
Tamas Sandor Harvard University

RO1-CA-23816 Optical Quality Effects on Lung Tumor Detection
Robert D. Moseley, Jr. University of New Mexico

R23-CA-23818 Image Reconstruction from Incomplete Projections
Robert M. Lewitt State University of New York at Buffalo

RO1-CA-24625 Diagnostic Accuracy and X-Ray Image Properties
Harold L. Kundel Temple University

RO1-CA-24806 Radiographic Imaging for Cancer Diagnosis
Kunio Doi University of Chicago

RO1-CA-24822 3-D Tomogram Viewer for Cancer Detection and Therapy
Brent S. Baxter University of Utah

R23-CA-25372 New Fluorescent X-Ray Tubes for Mammography
G. Allan Johnson Duke University

R01-CA-26327 Test Phantoms for Optimizing Mammographic Techniques
Leonard Stanton Hahnemann Medical College and Hospital

R01-CA-27823 A Scanning Equalization System for Chest Radiography
Donald B. Plewes University of Rochester

NON-RADIOLOGICAL IMAGING

R01-CA-17642 Thermography at Microwave and Millimeter Wavelengths
William R. Hendee University of Colorado Medical Center

R01-CA-19019 Ultrasound Mammography
Gilbert Baum Yeshiva University - Albert Einstein College of Medicine

R01-CA-23142 Thermographic Tumor Detection Enhanced by Microwave and Acoustic Energy
James E. Thompson University of South Carolina

R01-CA-23616 New Ultrasonic Methods for Breast Cancer Detection
John M. Reid Institute of Applied Physiology and Medicine

R01-CA-24085 Breast Diagnosis Quantitative Imaging by Ultrasound
James F. Greenleaf Mayo Foundation

R01-CA-24257 Breast Tumor Diagnosis by Computed Ultrasound
William Swindell University of Arizona

R01-CA-25323 Ultrasonic Computed Tomography of Breast Cancer
Paul L. Carson University of Colorado Medical Center

R01-CA-25634 Development of Ultrasonic Breast Tissue Phantom
James A. Zagzebski University of Wisconsin

R01-CA-25938 Scanning Acoustic Microprobe for Cancer Diagnosis
Frank E. Barber Harvard University

R13-CA-27038 Conference on Ultrasoft X-Ray Microscopy
Donald F. Parsons Electron Optics Lab.

R01-CA-27895 Transpelvic Ultrasound Evaluation of the Prostate
Aubrey M. Palestrant Beth Israel Hospital

R01-CA-27021 Heavy-Ion Radiography and Cancer
Cornelius A. Tobias University of California Berkeley

NUCLEAR MEDICINE

RO1-CA-03349 Potential Tumor or Organ Imaging Agents
Raymond E. Counsell University of Michigan at Ann Arbor

RO1-CA-15787 Radiotracers in the Diagnosis and Treatment of Cancer
Milo M. Webber University of California Los Angeles

RO1-CA-16861 Bifunctional Chelating Agents in Tumor Localization
Claude F. Meares University of California Davis

RO1-CA-17742 Radiological Localization of Human Tumors
James F. Primus University of Kentucky

RO1-CA-18153 Cancer Studies with New Radioactive Scanning Compounds
John S. Laughlin Sloan-Kettering Institute for Cancer Research

RO1-CA-19898 Synthesis of New 75SE Organ Specific Imaging Agents
Michael A. Davis Northeastern University

RO1-CA-22077 Zinc Radiopharmaceuticals for Ocular Melanoma Detection
Donald P. D'Amato University of Connecticut

RO1-CA-22578 Melanoma Delineating Radiopharmaceuticals
Ned D. Heindel Hahnemann Medical College and Hospital

PO1-CA-23417 Improved Tumor Imaging in Nuclear Medicine
Harrison H. Barrett University of Arizona

R23-CA-24344 Synthesis, Chemistry of Technetium Radiopharmaceuticals
Michael J. Clarke Boston College

RO1-CA-24597 Melanoma Detection with Radiopharmaceuticals
Harold L. Atkins Brookhaven National Laboratory

R23-CA-24879 Malignant Perfusion: Applied to Diagnosis and Therapy
Stuart W. Young Stanford University

RO1-CA-26371 Radiopharmaceuticals for Positron Tomography in Cancer
David R. Elmaleh Massachusetts General Hospital

RO1-CA-26968 Improved Tumor Imaging with New Radioactive Liposomes
Donald J. Hnatowich University of Massachusetts Medical School

RO1-CA-27252 Metabolism of Bone Scanning Radiopharmaceuticals
Charles D. Russell University of Alabama in Birmingham

RO1-CA-28105 An Electronically Collimated Gamma Tomography System
Manbir Singh University of Southern California

RO1-CA-28561 Development of Tumor and Organ Imaging Agents
Larry A. Spitznagle University of Maryland at Baltimore

MULTIPLE DISCIPLINES

RO1-CA-25582 Fluorescence Endoscopy and Photoradiation Therapy
Oscar J. Balchum University of Southern California

DESCRIPTION OF CURRENT CONTRACTS

The 68 contracts active during FY1980 are described in the following section.

CONTRACT RESEARCH SUMMARY

Title: Development of New Methods of Single Cell Separation

Principal Investigator: Dr. Michael Hercher and Mr. E. Robert Schildkraut
Performing Organization: Block Engineering
City and State: Cambridge, MA

Contract Number: N01-CB-74095
Starting Date: 8/17/77 Expiration Date: 12/29/80

Goal: To develop, test and install an apparatus for storing individual cells in the effluent of a flow cytometer in such a manner that any specified cell(s) may be subsequently retrieved for further examination. The immediate application of this apparatus will be in the validation of flow cytometric protocols for the early detection of cervical cancer.

Approach: The contractor shall develop techniques for depositing individual cells, which have passed through a flow cytometer, onto gelatin coated film and for uniquely marking their locations. The method of deposition shall be such that individual cell morphology is preserved, and cells can be subsequently restained and examined microscopically. The design of the deposition system should permit its ready adaptation to existing commercial and laboratory flow cytometers. In addition to marking the sequential locations of cells which have been analyzed in a flow cytometer, the marking system should be capable of indicating cell classifications. The contractor shall also design and develop a film reader which will facilitate the microscopic examination of cells deposited on film, and which will allow the rapid retrieval of specified individual cells. Final installation of the Cell Storage And Retrieval (CSAR) system (at a laboratory to be specified by the NCI), and training of its users, will be done by the contractor.

Progress: A film-based deposition and retrieval system has been developed and tested. Several hundred cells per second can be deposited and subsequently retrieved in the microscope film reader. The locations of individual cells have been permanently marked on the film during deposition, and there is provision for four different cell classifications. This system will be installed at a research facility in 1980, where it will be extensively tested on gynecological specimens.

Project Officer: Dr. Bill Bunnag
Program: Diagnosis Branch
Technical Review Group: Cytology Automation Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. Sadek K. Hilal
Performing Organization: Columbia-Presbyterian Hospital
City and State: New York, NY

Contract Number: N01-CB-43910
Starting Date: 6/30/74 Expiration Date: 12/29/79

Goal: To determine the value of computerized tomographic x-ray scanning in the detection and diagnosis of tumors of the brain, especially as compared to nuclide scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate the method by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of the normal brain. The number of patients available in this study will provide statistically significant information.

Progress: Patient enrollment and follow-up has been completed. Analysis and interpretation of accumulated data are being actively pursued. Results of the study will be published during 1980.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. D. Gordon Potts
Performing Organization: Cornell University Medical School
City and State: New York, NY

Contract Number: N01-CB-43980
Starting Date: 6/30/74 Expiration Date: 12/29/79

Goal: To determine the value of computerized tomographic x-ray scanning in the detection and diagnosis of tumors of the brain, especially as compared to nuclide brain scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate the method by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of the normal brain. The number of patients available in this study will provide statistically significant information.

Progress: The CT scan features of the brain metastatic lesions have been studied in detail. When these lesions are subdivided according to organ of primary involvement, the CT appearances are similar. No evidence was found that suggested that the organ of origin could be predicted from the CT appearances of a brain metastasis. Analysis of the data is continuing to determine whether the histology can be predicted from the CT appearances. Giant histological brain sections have been prepared on patients coming to autopsy to show the entire brain in the planes of the EMI sections; 121 cases have been studied in this manner. This will permit an accurate comparison of the x-ray CT scan views with the other studies.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Application of Flow System Methods of Cell Analysis and Sorting to Cancer Screening

Principal Investigator: Dr. Paul F. Mullaney
Performing Organization: DOE-Los Alamos Scientific Lab.
City and State: Los Alamos, NM

Contract Number: Y01-CB-60311
Starting Date: 5/1/76 Expiration Date: 7/31/80

Goal: Development of flow cytometric techniques applicable to clinical cytology in the context of cancer diagnoses.

Approach: During this terminal year, the contractor shall (1) test the sensitivity of the parabolic flow chamber developed at LASL, (2) evaluate the performance specifications of the LASL dual laser cell sorter, (3) maintain the LASL/NCI sorter at DCBD, and (4) design, fabricate and install a specified list of parts for the above mentioned LASL/NCI sorter.

Progress: Most aspects of items (3) and (4) are complete. The parabolic flow chamber has been remanufactured and is now ready to undergo evaluation. Several two fluorochrome combinations have been tested with model cell systems with the LASL two laser system. Yet to be accomplished is total evaluation of the two laser sorter with computer capability.

Project Officer: Dr. Bill Bunnag
Program: Diagnosis Branch
Technical Review Group: Cytology Automation Committee
FY 80 Funds: $2,929

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Application of Monocyte Function in Cancer

Principal Investigator: Dr. Ralph Snyderman
Performing Organization: Duke University
City and State: Durham, NC

Contract Number: NO1-CB-74121
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To determine the significance of abnormal monocyte function in humans with cancer.

Approach: The relationship between the clinical status of cancer patients and their monocyte chemotactic responsiveness in vitro is being determined. In addition, other parameters of phagocytic cell function are being examined to determine the specificity of depressed monocyte chemotaxis in cancer patients. The effects of serum, malignant effusions, tumor extracts, and urine from cancer patients on phagocytic cell function in vitro are being studied. The effect of tumor removal on phagocytic cell function is being examined.

Progress: The contractor has recently demonstrated that monocyte chemotactic responsiveness in vitro is depressed in approximately 60% of patients with cancer. Removal of the tumor by surgery or by immunotherapy reverses this defect. They have been able to demonstrate that monocyte phagocytosis and neutrophil chemotaxis are normal in cancer patients. This implies some degree of specificity for the monocyte chemotaxis defect. They have also found soluble inhibitors of monocyte chemotaxis in malignant effusions from cancer patients. Currently the contractor is measuring superoxide production and radiolabeled chemotactic factor binding by monocytes from patients with tumors. Patients are being restudied after tumor removal. They will determine if monocytes from patients with cancer have abnormal superoxide production or chemotactic factor binding. Correlation between monocyte function and prognosis in patients with cancer is being determined.

Significance to Cancer Research: The production, by neoplastic cells, of a substance which adversely affects monocyte function could be decisive in preventing the immunological elimination of cancer cells. Characterization of such a substance could have important implications with respect to both detection of cancer and therapeutic intervention.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Screening for Medullary Carcinoma of the Thyroid Using Calcitonin Radioimmunoassay

Principal Investigator: Dr. Samuel A. Wells, Jr.
Performing Organization: Duke University Medical Center
City and State: Durham, NC

Contract Number: N01-CB-63994
Starting Date: 6/15/76 Expiration Date: 6/14/81

Goal: To establish a periodic screening program of relatives of patients with medullary thyroid carcinoma (MTC).

Approach: A cohort of patients comprised of seven kindreds in which MTC is proven histologically will be established and be large enough to offer a statistically significant number of family members. Those family members will be screened for serum calcitonin levels by radioimmunoassay methods in order to detect MTC in its earliest stage of development. A long-term follow-up program will be conducted on these families to determine the natural history of the disease and the effectiveness of surgical and possibly chemical therapy.

Progress: Our study of approximately 750 subjects from seven kindreds with multiple endocrine neoplasia, type II (medullary thyroid carcinoma (MTC), pheochromocytoma and parathyroid hyperplasia), has completed its fourth year. We have demonstrated that the combined infusion of calcium gluconate (2 mg/kg/1 min.) followed by pentagastrin (0.5 μg/kg/5 sec.) is the preferred method of stimulating calcitonin (CT) release from MTC cells. Patients with occult MTC who are diagnosed biochemically following provocative testing are younger, have a lower incidence of regional node metastases, and have a lower incidence of elevated plasma CT levels following provocative testing postoperatively. In patients who have borderline elevated calcitonin levels following provocative testing (250-1,000 pg/ml), we selectively catheterized the inferior thyroid vein. Plasma calcitonin levels have been determined following provocative testing. Significantly elevated levels (greater than 1,500 pg/ml) compared to peripheral levels indicate the presence of MTC as has been confirmed by subsequent surgery. Patients without MTC have not been demonstrated to have elevated calcitonin levels and selectively catheterized the inferior thyroid veins following provocative testing. We have evaluated Adriamycin, Cis platinum and Streptozotocin in the treatment of patients with metastatic MTC. In only one of five patients treated with Adriamycin (maximum dose 350 to 1,000 mg/m^2) has there been evidence of tumor regression, indicated by a decrease in size of a metastatic soft tissue nodule and concomitant decreasing levels of plasma calcitonin. No patients responded to Cis platinum therapy or to Streptozotocin therapy.

Project Officer: Mr. Louis P. Greenberg
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $106,000

CONTRACT RESEARCH SUMMARY

Title: Data Management System and Statistical Support for NCI Serum Panel

Principal Investigator: Dr. Lee Richman and Dr. C. M. Dayton
Performing Organization: Ebon Research Systems
City and State: Silver Spring, MD

Contract Number: NO1-CB-74210
Starting Date: 8/8/77 Expiration Date: 8/7/81

Goal: To provide data management and statistical programming support for research projects being conducted by the Diagnosis Program in order to determine which serum tests are best able to detect early stages of cancer.

Approach: To perform statistical analyses of data from NCI serum panel evaluations and to prepare summary reports of the results.

Progress: Approximately two serum panels are received per month, and, in general, the following statistical analyses are supplied for each panel: 1) plots, such as histograms, so that the distributions across clinical groups can be compared. In most instances, an analysis of variance accompanies each plot. 2) Chi-square (2x2) tables are formed for a variety of clinical comparisons, and accompanying each are the chi-square value, its associated two-tailed probability, and the sensitivity, specificity, and misclassifications of the comparison. 3) When appropriate, McNemar chi-square statistics to test whether or not the rates of positive classifications are the same for two assays. 4) For multiple assays, a Discriminant Analysis is often used to combine information from the assays into a single prediction function. 5) Gail-Green procedures for locating optimal cutting scores. 6) Scattergrams for pairs of assays. Other statistical tests are done as appropriate.

Significance to Cancer Research: Since it is important to diagnose and treat cancer as early as possible, it is important to have a diagnostic and screening tool that would enable one to detect early cases of cancer. These analyses will provide information as to the degree of success with which researchers have developed such tools.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $59,122

CONTRACT RESEARCH SUMMARY

Title: Immunodiagnostic Markers for Breast Carcinoma

Principal Investigator: Dr. Rajender K. Chawla
Performing Organization: Emory University
City and State: Atlanta, GA

Contract Number: N01-CB-84308
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: To evaluate the ability of the crossed immunoelectrophoresis (CIEP) technique to the immunodiagnosis of breast cancer by monitoring the glycoprotein EDC1, the principal component of "cancer proteinuria."

Approach: Pure EDC1 and IATI (inter-alpha-trypsin inhibitor), and high titer antiserum to EDC1 will be prepared. A standardized method for assaying EDC1 will be developed. The CIEP method will be used to measure plasma/urine levels of EDC1 and IATI in normal women and those with benign diseases and breast carcinoma. EDC1 levels in plasma/urine will be monitored preoperatively in patients with a localized breast mass and postoperatively.

Progress: EDC1, found in the urine of a patient with acute myelocytic leukemia, has been isolated and identified by this group as a degradation product of inter-α-trypsin inhibitor (IATI), a normal plasma protein. A specific antiserum was raised to the EDC1 and a radioimmunoassay was developed. EDC1 has now been shown to be excreted in at least seven different types of cancer, more so when the disease is metastatic than when localized.

The contract has devised several methods for quickly and easily separating EDC1 from IATI in the serum so that it can be measured separately. Clinical evaluation of patients with breast cancer compared to those with benign breast disease and normal controls is now underway.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $83,567

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. David O. Davis
Performing Organization: The George Washington University
City and State: Washington, DC

Contract Number: N01-CB-43981
Starting Date: 6/30/74 Expiration Date: 12/29/79

Goal: To determine the value of computerized tomographic x-ray scanning in the detection and diagnosis of tumors and other lesions of the brain, especially as compared to nuclide brain scanning, angiography, pneumoencephalography.

Approach: Five cooperating institutions will evaluate the method by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the clinical probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of the normal brain. The number of patients available in this study will provide statistically significant information.

Progress: Patient enrollment and follow-up has been completed. A total of 525 patients were enrolled: 376 were in the first group, 86 were in the second group, and 63 normals were examined. Of the total, 185 patients had brain tumor(s). Analysis of the data is being pursued at all centers. Clinical details and taped images are being shared by all centers, with individual centers coordinating separate projects.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of Immunodiagnostic Tests for Cancer

Principal Investigator: Dr. Adly N. Ibrahim
Performing Organization: Georgia State University
City and State: Atlanta, GA

Contract Number: NO1-CB-84267
Starting Date: 2/1/78 Expiration Date: 1/31/81

Goal: Immunodiagnosis of cervical and head and neck cancers.

Approach: Antisera are prepared in rabbits against cervical cancer tissues. Elimination of antibodies against normal tissues and normal human sera is carried out by various methods. These antisera will be used for the detection of circulating tumor-associated antigens (C-TAA) in sera of patients with cervical and head and neck cancers. Serum controls including those from normal individuals and from patients with benign diseases of these sites will be used.

Progress: The contractor has prepared hyperimmune sera against partially purified cervical cancer-associated antigens. Tumor-associated antigens were demonstrated in cervical cancer tissue and in the sera of patients with cervical and head and neck cancers. Testing a coded panel of sera from the NCI Mayo Serum Bank, the contractor showed significant results with high specificity but low sensitivity. To improve specificity, attempts to obtain highly purified anti-CaCx sera are being made by (a) purification of antisera prepared against crude or partially purified CaCx, and (b) purification of CaCx TAA to be used as immunogen for rabbits. For isolation of purified TAA and anti-TAA, methods of purification of proteins will be carried out including salt fractionation, gel filtration, ion exchange, isoelectric focusing, and affinity chromatography. To increase sensitivity, the purified antisera will be used to explore the possibility of employing the more sensitive and quantitative serological test "enzyme-linked immunosorbent assay" (ELISA) for the detection of C-TAA. Each purified fraction of CaCx TAA will be evaluated for its ability to: (1) elicit antibodies capable of detecting CaCx TAA and C-TAA in sera of cervical and head and neck cancer patients, and (2) produce highly specific antibodies in rabbits, i.e., capable of reacting only with CaCx TAA and C-TAA but not with normal tissues, normal sera, or sera from patients with benign gynecological or head and neck diseases.

Significance to Cancer Research: Results obtained so far suggest the possibility of developing serological tests for diagnosis, prognosis, and monitoring patients with cervical and head and neck cancers.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $78,454

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Ovarian Carcinoma in Asymptomatic Women

Principal Investigator: Dr. Malaya Bhattacharya
Performing Organization: Health Research, Inc.
City and State: Buffalo, NY

Contract Number: N01-CB-64013
Starting Date: 6/30/76 Expiration Date: 12/31/79

Goal: The development of sensitive immunoassay procedures for early diagnosis of ovarian cancers and serial monitoring of patients undergoing therapy for ovarian cancers so that treatment can be more effective.

Approach: The ovarian cancer-associated antigens/enzymes will be isolated from solid and cystic cystadenocarcinomas as well as ascitic effusions obtained from patients with ovarian carcinomas. The antigens and/or enzymes will be purified by using standard biochemical procedures, as well as affinity columns. The purified material will be used to raise high titer, high affinity immune sera in rabbits. Using the highly specific antiserum and the purified antigens (or enzymes), sensitive radioimmunoassay (RIA) procedures will be developed for the determination of antigen (or enzyme) levels in the sera of ovarian cancer patients and suitable controls.

Progress: We identified six ovarian cancer-associated antigens designated OCAA, OCAA-1,2,3,4 and 5, which could be found in ovarian tumors but not in normal ovaries or normal adult serum. OCAA is a unique high molecular weight glycoprotein apparently confined to cystadenocarcinomas of the ovary. High levels of circulating OCAA were detected by RIA in sera of ovarian cancer patients. OCAA-1 is a pregnancy-associated antigen present in other tumor types. We purified OCAA-1 to apparent homogeneity with the goal of preparing high titered antisera and developing radioimmunoassay. OCAA-1 is immunologically related to the ovarian cancer antigen OVC-1 described by Immamura et al. OCAA-2 is a fetal lung-associated antigen which is elevated in about 40% of ovarian and colonic adenocarcinomas. OCAA-3 and OCAA-4 are both very high molecular weight glycoproteins present in mucinous variety of ovarian tumors. OCAA-3 is also present in normal gastrointestinal tissues and cross-reacts wih M_4 antigen of Bara et al. OCAA-5 is a CEA-like antigen characteristic of mucinous ovarian cysts of the mixed intestinal endocervical type only. Immunohistochemical studies of OCAA-3, -4, and -5 might provide better criteria for immunological classification of mucinous ovarian tumors. Of all the enzymes studied, UDP-Galactose: glycoprotein galactosyltransferase is the best enzyme marker for ovarian adenocarcinomas. Serum levels of this enzyme reflected the clinical status of the patient with respect to tumor progression and tumor burden. Purificaton of galactosyltransferase is underway to develop a radioimmunoassay for this enzyme.

Project Officer: Mr. Louis P. Greenberg
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Tumor-specific Antigens in Diagnosis and in the Management of Cancer Patients

Principal Investigator: Dr. T. M. Chu
Performing Organization: Health Research, Inc.
City and State: Buffalo, NY

Contract Number: NO1-CB-33858
Starting Date: 6/25/73 Expiration Date: 10/24/80

Goal: To study the relationship of CEA in the evaluation of recurrence and survival for cancer patients with curative surgery.

Approach: Examine the prognostic value of the preoperative and follow-up CEA in relation to other preoperative variables; study the distribution profiles of CEA at surgery, during follow-up period, and at recurrence; implement a plan for patients whose risk of recurrence is greatest based upon preoperative and serial CEA; develop predictive models which integrate the CEA levels with other significant prognostic variables to determine the probability of recurrence in order to recommend adjuvant therapy.

Progress: An analysis of long-term follow-up CEA data and baseline data is under way on 74 patients with colorectal cancer and on 130 patients with lung cancer who underwent resections for cure. To date, in 74 colorectal cancer patients, 33 had recurrence of disease and 29 died. Preoperative CEA was found to be correlated with Dukes' classification, and had significant prognostic value between 9 and 18 months post surgery. Thereafter, Dukes' classification was a more significant predictor of disease recurrence. CEA was often elevated in anticipation of recurrence, sometimes as early as one year prior to recurrence. Among patients experiencing recurrence, although forewarned by CEA elevations, no significant correlation was found between time interval and risk of recurrence. The correlation of CEA elevations alone with the time of recurrence was not statistically significant enough to suggest a second-look surgery without other supporting evidence. At present, in collaboration with NCI, a time-dependent Cox statistical model is being developed to re-investigate the relationship between follow-up CEA and disease recurrence. In patients with resectable lung carcinoma, CEA has its more precise value in distinguishing, at an early date (sometimes at surgery), cured patients from those who would fail subsequent to surgical resection because of recurrent disease. Adjuvant therapies could thus be recommended for those patients with high risk for recurrence. Additional accomplishment was the isolation of a CEA-binding globulin from normal serum by CEA affinity chromatography.

Significance to Cancer Research: CEA is a laboratory assay which can be used as an aid in the management of certain cancer patients.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $36,130

CONTRACT RESEARCH SUMMARY

Title: Immunologic Markers Applicable to Cytology Automation

Principal Investigator: Dr. Laure Aurelian
Performing Organization: Johns Hopkins University
City and State: Baltimore, MD

Contract Number: NO1-CB-74170
Starting Date: 7/18/77 Expiration Date: 7/17/81

Goal: To study qualitative and quantitative antigenic changes in premalignant and malignant cells.

Approach: The contractor shall isolate HSV antigens: (i) total viral antigens, (ii) AG-e, and (iii) fractions of AG-e antigen, and prepared corresponding purified antisera. The antisera will be used to stain human gynecological materials in indirect immunofluorescence. The sensitivity and specificity of these antisera, in terms of their ability to discriminate premalignant and malignant cells from normal cells, will be determined. Immunological and biochemical characterization of the antigens will be done.

Progress: The preparation of antigens from HSV infected cells and their purification has proceeded as scheduled. AG-e purified by crossed immunoelectrophoresis has been resolved by SDS-acrylamide gel electrophoresis into two component proteins designated ICP 12 (M.W. 140,000) and ICP 14 (M.W. 130,000). ICP 12 and ICP 14 were purified to radiochemical homogeneity by SDS-acrylamide gel electrophoresis. Antisera were prepared against "pure" AG-e, ICP 12 and ICP 14 and their reactivity in a wide variety of serologic assays was determined. Antisera to ICP 12 react with soluble antigenic mixtures from HSV-2 infected cells in crossed immunoelectrophoresis and both anti-ICP 12 and ICP 14 sera neutralize virus infectivity in presence of antiglobulin and stain virus-infected cells in indirect immunofluorescence. ICP 12 and ICP 14 appear to be envelope proteins as evidenced by: (i) the adsorption of the reactivity of anti-ICP 12 and ICP 14 sera with HSV-2 (G) virions but not with "mock" virus preparations, (ii) their ability to neutralize HSV-2 in presence of anti-IgG, and (iii) the resolution of a protein with the relative mobility of ICP 14 in virions the surface of which is iodinated with lactoperoxidase. In indirect immunofluorescence, antisera to "pure" AG-e, ICP 12 and ICP 14 specifically stain exfoliated atypical cells. Normal cells are not stained. The promise of the immunofluorescent staining procedure is presently under investigation in a double-blind study designed to compare immunofluorescent marking of specimens by antiserum to "pure" AG-e (apparently the best marker and routine manual screening and microscopic examination). Specimen by specimen and cell by cell comparisons are performed. To date, very good correlation (80-93.8%) is observed between staining with anti-"pure" AG-e serum and routine manual screening.

Project Officer: Dr. Bill Bunnag
Program: Diagnosis Branch
Technical Review Group: Cytology Automation Committee
FY 80 Funds: $77,450

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer Control: Detection and Treatment

Principal Investigator: Dr. John K. Frost
Performing Organization: Johns Hopkins University
City and State: Baltimore, MD

Contract Number: N01-CB-45037
Starting Date: 9/1/73 Expiration Date: Indefinite

Goal: To determine the effect of early detection of lung cancer by sputum cytology followed by surgical removal of the tumor on mortality from this disease.

Approach: The study population consists of males who, at the time of enrollment, were 45 years or older, and had smoked a pack or more of cigarettes a day. Individuals responding to direct mailing were randomly assigned to two groups: the test group receives an annual chest x-ray, an annual sputum induction plus sputum cytologic examination every four months; the control group receives an annual chest x-ray only. Subjects suspicious or positive for cancer are carefully evaluated including localization of the cancer by fiberoptic bronchoscopy; cancers are removed surgically when possible. All subjects will be followed for at least 5 years to determine actuarial survival.

Progress: A total of 10,828 men have been enrolled in this project, of which 5,407 were randomized into an "X" Control Group and 5,421 into a "CX" Study Group. To date, 385 have died, while 851 have withdrawn from the study, and 114 have moved from the area. Currently, 8,909 remain as active participants; 4,462 in the "X" Group and 4,447 in the "CX" Group. At the initial screening 74 cancers were detected (39 in the X group and 35 in the CX group) for a detected prevalence of 7.1 per 1,000. Following an initial negative screening, a total of 143 cancers appeared (74 in the X group and 69 in the CX group) for an incidence of 4.4 per 1,000. In the CX group there were 12 cases detected (17%) in Stage 0 and 24 (35%) in Stage 1. Twenty-six additional cases occurred in the CX group between screening procedures. In the X group no cases were detected in Stage 0 and 27 (36%) Stage 1. Twenty-four additional cases occurred in the X group between screenings. Among the 69 incidence cases detected in the CX group 34 (49%) were resected; 32 (43%) of the 74 incidence cases detected in the X group were resected. Two hundred seventeen total lung cancers have appeared in the screened population, 113 in the X group and 104 in the CX group. Among the 113 cases of lung cancer found in the X group 59 (52%) have died; 45 (43%) of the 104 lung cancer cases in the CX group have died. The observed rates of lung cancer mortality are 45 per 10,000 person-years in the X group and 26 per 10,000 person-years in the CX group.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $796,164

CONTRACT RESEARCH SUMMARY

Title: Immune Assays for Enzymes and Isozymes in Cancer

Principal Investigator: Dr. Joel Shaper
Performing Organization: Johns Hopkins University
City and State: Baltimore, MD

Contract Number: NO1-CB-84260
Starting Date: 9/22/78 Expiration Date: 9/21/81

Goal: Development of immunodiagnostic assays for cancer using an enzyme or isoenzyme as an antigen and looking for a correlation of enzyme/isozyme serum levels with tumor burden.

Approach: Evaluate the immunodiagnostic significance of serum levels of UDP-galactosyltransferase in human breast and ovarian carcinoma.

Progress: Antisera directed against affinity-purified bovine UDP-galactosyl-transferase have been prepared in rabbits and partially immunologically characterized. These antisera have relatively high titered precipitating antibody to bovine galactosyltransferase as assessed by the Ouchterlony double diffusion technique and inhibition of enzymatic activity. The antisera are highly cross-reactive with human UDP-galactosyltransferase found in both normal serum and ascitic fluid obtained from a patient with ovarian cancer as determined by direct precipitation and inhibition of enzymatic activity. This cross-reactivity has been exploited for the development of a sensitive and specific radioimmunoassay for human serum UDP-galactosyltransferase with a detection level of 0.1-1.0 nanograms in the assay mixture.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $70,150

CONTRACT RESEARCH SUMMARY

Title: Application of Digital Image Processing Techniques to Cytology Automation

Principal Investigator: Dr. Kenneth R. Castleman
Performing Organization: JPL--California Institute of Technology
City and State: Pasadena, CA

Contract Number: Y01-CB-70314
Starting Date: 9/20/77 Expiration Date: 9/30/81

Goal: Identification of the best performing image analysis algorithms and estimations of the expected performance and cost per specimen of an automated cervical cancer pre-screening system using these algorithms.

Approach: The contractor shall determine whether or not the current state of the art in pattern recognition is adequate to support the development of an economically viable cytological screening instrument based on single cell classification. Specimen preparation protocol will be optimized so as to produce acceptable single cells for digitization. A large number of digitized images will be accumulated in a library for a subsequent feature extraction program to produce a feature data base. A series of classification experiments will be run on the feature data base to select the subset of cell measurements that yields the best overall performance. An analysis will produce estimates of the cost and performance using these techniques in practice.

Progress: A new specimen preparation protocol has been developed. Over 10,000 digitized cell images were acquired and classification algorithms were developed and tested. The results of this portion of the study indicate that the cell classification algorithms chosen can operate with error rates that are adequate for routine screening. The second phase of the study has been initiated. This phase will evaluate the performance of the cell classification algorithms on cells selected and segmented without human intervention.

Project Officer: Dr. Bill Bunnag
Program: Diagnosis Program
Technical Review Group: Cytology Automation Committee
FY 80 Funds: $216,215

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of Immunodiagnostic Tests for Cancer

Principal Investigator: Dr. John Fenimore Cooper
Performing Organization: Kaiser Foundation
City and State: Oakland, CA

Contract Number: NO1-CB-74169
Starting Date: 9/16/77 Expiration Date: 9/15/80

Goal: Evaluate an immunodiagnostic test for prostatic cancer.

Approach: Accumulate and assess clinical oncologic data for prostatic cancer with a solid phase radioimmunoassay technique for prostatic acid phosphatase (RIA-PAP); continue development of the RIA-PAP technique as a screening technique in the early diagnosis of prostatic cancer utilizing a large random population of elderly males.

Progress: The contractor has completed the evaluation and quantitation of prostatic acid phosphatase by radioimmunoassay on all clinical samples. These samples fall into the following categories: controls of varying ages, 12,320 samples; men with benign prostatic hypertrophy, 350 samples; men with cancer of the prostate, 4,500 samples taken both before and after therapy; and men with other histologic types of cancer, 200 samples. In the last 6 months the data have been entered on computer cards and analysis is now under way at the UCLA computer facility.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Applications of Antibodies to Melanoma

Principal Investigator: Dr. Peter Hersey
Performing Organization: Kanematsu Institute
City and State: Sydney, Australia

Contract Number: NO1-CB-74120
Starting Date: 9/30/77 Expiration Date: 9/29/81

Goal: Determine whether there is a correlation between the clinical course of melanoma growth and levels of serum antibodies.

Approach: Employ lymphocyte-dependent cytotoxic antibody (LDA) assays.

Progress: During the first 27 months of this study, 267 patients with localized melanoma were studied. Of these, 130 had LDA activity detected against allogeneic cultured melanoma cells. In 87 patients a rise in LDA activity was noted after surgical removal of the melanoma followed by a gradual delay to undetectable levels by 2-3 months. Eleven patients had detectable antibody to melanoma cells before removal of melanoma which remained unchanged after surgery. Ten of the latter were multiparous women. Of the 169 patients with no antibody detectable in their sera, 37 had LDA activity detectable in the IgG fractions of sera obtained by gel filtration of acidified 'unblocked' sera. Sera from 14 of 85 patients with non-melanoma carcinoma had LDA activity to allogeneic melanoma cells. All but one of the sera from this latter group of patients also reacted with allogeneic cells from cell lines established from breast and bladder carcinoma and the Chang liver cell line and were not specific for melanoma. This contrasted with the results of studies on the melanoma patients in that 103 of the 130 positive sera did not react with cells from non-melanoma cultures. Studies on the specificities of these sera are in progress. Sixty-four of the patients entered into the study have relapsed and preliminary analysis of the correlation between clinical progress and antibody status is in progress. Patient accrual will cease after entry of 300 patients into the study.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $53,222

CONTRACT RESEARCH SUMMARY

Title: Study of Innovative Techniques to Facilitate Passage of Colonoscope to the Cecum

Principal Investigator: Dr. John A. Coller
Performing Organization: Lahey Clinic Foundation
City and State: Boston, MA

Contract Number: N01-CB-74212
Starting Date: 9/26/77 Expiration Date: 9/25/80

Goal: To develop a means for improving early detection of cancer on endoscopically approachable surfaces of the colon in order to improve life expectancy.

Approach: A colonoscope advancing device will be developed to enhance the ease and speed of passage of currently available colonoscopes. Modeling studies and bench testing will be conducted during the initial year to determine which of several possible designs may be feasible. Further development of the colonoscope advancing device design will be carried out during the second year aided by physiological studies made to characterize as many physical parameters of the human colon as possible. Suitable devices will be given preliminary clinical tests during the latter part of the second year. After further optimization the clinical prototype propulsive devices will be tested by clinical trials in the third year.

Progress: During the first year, efforts were directed towards defining and measuring physiologic characteristics of colon function that relate to the intubation problem. From these observations, together with a mathematical modeling approach, various bench models were developed for testing discrete aspects of the problem. The various techniques in the construction of fibroelastomeric structures were developed. During the second year, fibroelastomeric structures of various configurations were constructed and submitted to both bench and animal testing. A major problem which became apparent at this point was the disparity between the various bench models and the more compliant animal colon. Consequently, the major effort during year three has been to use the animal preparation as the main evaluatory system with fibroelastomeric devices that are designed to accept rather than combat the compliant nature of the colon. At present, suitable fibroelastomeric constructions are being prepared for evaluation in humans.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Development and Evaluation of New Stains and Other Optical Markers Useful for Cytopathologic Specimens in Suspension

Principal Investigator: Dr. Brian H. Mayall
Performing Organization: Lawrence Livermore Laboratory
City and State: Livermore, CA

Contract Number: Y01-CB-40300
Starting Date: 6/1/74 Expiration Date: 7/31/80

Goal: To develop and evaluate stains that can be used in flow cytometry to differentiate normal from dysplastic and malignant gynecologic specimens.

Approach: The contractor will complete implementation of the Livermore dual beam sorter, utilizing computer-controlled sorting and computer-based acquisition, display, and analysis of data. DNA-DNA stain pairs will be used to probe differences in chromatin structure between normal and neoplastic cells. Other stain pairs will be evaluated for their diagnostic potential.

The dual laser sorter and multivariable analysis techniques will be used to discriminate between normal and dysplastic or malignant specimens. The analysis will be verified by sorting subpopulations of cells for cytomorphologic characterization.

Progress: Cellular DNA content, as probed by chromamycin A3 stain uptake, and cell size, as indicated by orthogonal light scatter, enable dysplastic and malignant cells, when present, to be detected by flow cytometry. But false alarms--clumps of two more normal cells being sensed by the system as single objects--has limited the diagnostic power of flow cytometry. Brief incubation of the specimen with DNase followed by centrifugal elutriation reduces the incidence of false alarms. Dual beam cytometry utilizes three variables: either chromomycin A3 - Hoechst 33258 energy transfer fluorescence for chromatin structure or chromomycin A3 and stilbene isothiocyanate disulfonic acid fluorescence for DNA and protein, together with orthogonal light scatter for cell size, to identify subpopulations of cells in gynecologic specimens. Dual beam sorting and cytomorphologic characterization verifies that, in dysplastic and malignant specimens, these subpopulations are greatly enriched in neoplastic cells. Specimens are diagnosed based on analysis of the three-variable flow cytometric data. Results on an initial set of 24 specimens indicate low specimen misclassification rates. Two of 12 negative specimens and 1 of 12 positive specimens were misclassified when compared to conventional cytomorphologic diagnosis; the estimated error rates from this limited trial are 17 percent false positive and 8 percent false negative. It is necessary to analyze many more specimens in order to establish the diagnostic utility of this approach. These studies are continuing.

Project Officer: Dr. Bill Bunnag
Program: Diagnosis Branch
Technical Review Group: Cytology Automation Committee
FY 80 Funds: $76,000

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. Paul F. J. New
Performing Organization: Massachusetts General Hospital
City and State: Boston, MA

Contract Number: NO1-CB-43983
Starting Date: 6/30/74 Expiration Date: 12/29/79

Goal: To determine the value of computerized tomographic x-ray scanning in the detection and diagnosis of tumors of the brain, especially as compared to nuclide brain scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate the method by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: Data accumulation having been completed, within recent months, a series of reports dealing with the overall results and a variety of component parts of the study have been completed or are nearing completion. A detailed report on the 164 meningioma cases in the study has been completed and should be published shortly in conjunction with reports from the other institutions involved in the study.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator:	Dr. Hillier L. Baker, Jr.
Performing Organization:	Mayo Foundation
City and State:	Rochester, MN

Contract Number:	N01-CB-43982		
Starting Date:	6/15/74	Expiration Date:	12/29/79

Goal: To determine the value of computerized tomographic x-ray scanning in the detection and diagnosis of tumors of the brain, especially as compared to nuclide brain scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate the method by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of the normal brain. The number of patients available in this study will provide statistically significant information.

Progress: Patient enrollment has been completed. Analysis and interpretation of accumulated data are being actively pursued. Four of seven papers detailing results have been completed and will appear in an early issue of RADIOLOGY.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Mayo Lung Project for Detection and Localization of Early Lung Cancer

Principal Investigator: Dr. Robert S. Fontana
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: NO1-CB-53886
Starting Date: 10/01/74 Expiration Date: Indefinite

Goal: To test new methods of diagnosis of early lung cancer and to assess survival of patients with lung cancer detected by these methods.

Approach: A study population of 9,223 subjects was obtained following initial screening of 11,001 non-volunteers. Candidates for the study were Mayo Clinic outpatients who were men, 45 years old or more, smoking one pack of cigarettes or more daily. None of those accepted into the study had a history or suspicion of respiratory cancer on entry into the Clinic; all had a life expectancy of at least 5 years, and all were judged capable of tolerating pulmonary resection (at least lobectomy). Initial screening, consisting of chest x-rays, 3-day sputum cytology tests and lung health questionnaires, yielded 87 unsuspected ("prevalence") lung cancers, 16 detected by cytology, 54 by x-ray, and 17 by both tests. Radiographically "occult" cancers were localized fiberbronchoscopically. Subjects who had "negative" initial screens were randomized into a close surveillance group, rescreened every 4 months, and a standard surveillance ("control") group advised to undergo rescreening once a year. Post-surgical AJC stage I lung cancer patients are also being evaluated, as are hematoporphyrin derivative (HpD) and cryotherapy as techniques for "mapping" and treating carcinoma in-situ.

Progress: As of October 1, 1979, both the 4-monthly surveillance group and the "control" group had been observed more than 20,000 man-years, and 213 new ("incidence") cases of cancer of the respiratory tract had been detected, 51 involving the upper airway and 162 the lungs. In the 4-monthly surveillance group there were 98 new lung cancers (12 of which were detected by cytology only) and 41 lung cancer deaths. Early survival data in this group are encouraging. In the control group there were 57 new lung cancers and 45 lung cancer deaths, with a tendency toward more traditional lung cancer survivorship. Once lung cancer has been detected, survival in the 4-monthly surveillance group is better than among the controls. No significant decrease in total death rate from lung cancer has been observed in the close-surveillance group, but further observation and prolonged follow-up are essential before conclusions and specific recommendations will be possible. As "incidence" cases continue to accrue more detailed analyses of data are becoming feasible. Attention is currently being directed towards evaluation of the results of screening by cell type of tumor and by modality of detection.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $710,115

CONTRACT RESEARCH SUMMARY

Title: Usefulness of Carcinoembryonic Antigen in the Diagnosis of Bowel Cancer

Principal Investigator: Dr. Vay Liang W. Go
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: N01-CB-23854
Starting Date: 6/15/72 Expiration Date: 6/14/81

Goal: (a) Measure CEA levels in blood and other specimens stored at NCI-Mayo Clinic Tumor Plasma Bank (CB-84258), Mayo Lung Cancer Project (NCI-CB-22000) and other contracts with the Immunodiagnosis Program of the Division of Cancer Biology and Diagnosis; (b) continue to collect and provide colonic malignant tissue for different investigators in projects dealing with carcinoembryonic antigens; (c) to complete the work done over the last five years in the continuation of the evaluation of CEA as a biologic marker in patients undergoing chemotherapy, radiotherapy, and immunotherapy of advanced gastrointestinal cancer.

Progress: (a) Progress related to the contract's service function activity. Serum or plasma levels have been determined in 8,211 specimens from NCI Serum Plasma Bank including those sent in from other investigators. These samples were run at 20% of the commercial cost. The comparative value of CEA to other tumor-associated antigens was done with other investigators from other institutions. Statistical analyses of the data over the last three years were included in the Workshop on Immunodiagnosis of Human Cancer Statistical Analyses, Part II. Results and statistical analyses are being performed on coded serum panels sent to many investigators with potentially useful diagnostic tests for cancer.

(b) Progress report on the evaluation of CEA as a biological marker. The collection of data has not been completed for the project in the evaluation of CEA as a biological marker in patients undergoing chemotherapy, radiotherapy, and immunotherapy for advanced gastrointestinal cancer and also in patients following surgical resection of gastrointestinal cancer with curative intent and lung cancer.

Project Officer: Mr. Louis P. Greenberg
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $54,900

CONTRACT RESEARCH SUMMARY

Title: Reconstruction Algorithms for Dose Reduction in X-Ray Computed Tomography

Principal Investigator: Dr. Richard A. Robb
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: NO1-CB-84235
Starting Date: 9/29/78 Expiration Date: 9/28/81

Goal: To develop and evaluate new and/or improved reconstruction methods directed toward reducing the x-ray dosage required to obtain diagnostically accurate information from CT scanning procedures used in cancer diagnosis.

Approach: Various approaches will be investigated for their possible contributions to improved image reconstruction with reduced x-ray dosage. These will include studies for tailoring and optimizing convolution, least square, and iterative algorithms for low x-ray dose data; use of a priori information; noise reduction techniques; variance maps for regional reconstruction; minimizing range, number, and field of view; non-linear spatial smoothing, and optimizing algorithms based on multiplier methods, penalty functions, and multiple criteria optimization. The methods will be tested on mathematical simulations and on patient CT scan data. Results will be evaluated using standard image quality measurement criteria and by psychophysical (ROC) studies.

Progress: Different versions of the convolution method were compared; for example, use of linear versus cubic spline interpolation and divergent beam algorithms with and without rebinning. Software for x-ray projection generation has been extended and special geometries of data collection have been studied. Positivity constraints have been investigated in iterative and convolution type algorithms and their effect on reconstruction accuracy and speed of convergence. The maximum entropy algorithm has been applied to simulated low dose CT data containing complex lesions in uniform fields. Routines for variance and covariance mapping have been implemented and will be used with selective smoothing for evaluating regional improvements in reconstructions. Algorithms for limited field of view and range of views have been developed. Studies with limited number of views indicate that high contrast lesions are detectable and measurable at very low x-ray doses. A thorough survey of the field of optimizing algorithms has been prepared. Extensions of known methods for quadratic optimization have been produced. Results with selective non-linear smoothing techniques indicate significant improvement in image signal-to-noise with potential for improvement in diagnostic quality. Uniform water bath scan fields with superimposed complex lesions have been produced to aid in the training of the observers.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $121,306

CONTRACT RESEARCH SUMMARY

Title: Maintenance of the NCI Serum Bank

Principal Investigator: Dr. Allan J. Schutt
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: NO1-CB-84258
Starting Date: 9/30/78 Expiration Date: 11/30/80

Goal: To establish and maintain a bank of sera from patients with cancer, with benign diseases and from normal individuals.

Approach: Make necessary serum samples available for evaluation of immunodiagnostic tests for cancer. Serve as a central facility for storage of serum and plasma specimens collected by other contractors in the Tumor Immunology-Immunodiagnosis Program.

Progress: In 1979, 11,340 vials of sera were collected by the central facility. Another 4,539 were collected from the Philadelphia Geriatric Screening Project. A total of 10,128 vials were shipped out. Presently 65,935 vials from the central facility and 160,433 vials from various screening projects are stored at Mayo.

Significance to Cancer Research: This serum bank will provide sera, in sufficiently large quantities and with adequate controls, to efficiently and rapidly evaluate potential immunodiagnostic tests for cancer.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Use of Cross-reacting Microbial Antigens

Principal Investigator: Dr. Christine H. Granatek
Performing Organization: M.D. Anderson Hospital
City and State: Houston, TX

Contract Number: NO1-CB-74122
Starting Date: 9/16/77 Expiration Date: 9/15/80

Goal: To study the distribution of an isolated microbial cross-reactive antigen in human leukemia and melanoma, produce specific antisera, and develop a radioimmunoassay for detection of circulating cross-reactive antigen.

Approach: Initial screening of tumor cells by immunofluorescence and of cancer patients' sera by a solid phase RIA, to detect reactivity with antimicrobial sera. Isolation of cross-reactive components by affinity chromatography. Specificity testing by absorption studies and cross-testing in RIA, with normal donor and benign disease patient control.

Progress: The primary tumor-associated BCG cross-reactive component isolated by affinity chromatography of AML and melanoma extracts was a glycoprotein of approximately 70,000 daltons in molecular weight, which also had fetal cross-reactivity. This component has widespread immunogenicity in leukemia and melanoma patients. Monospecific (rabbit) antibody has been produced against antigen isolated from mouse fetal liver by its affinity to antibody originally made against BCG cross-reactive antigen from an eluate of human AML blast cells. This antibody is reactive with sera from active disease leukemia and melanoma patients' sera, but not normal human serum, by counterimmunoelectrophoresis, and is being utilized for a quantitative radioimmunoassay to monitor sequential levels of antigen in the patients' sera.

Significance to Cancer Research: Assays based on either detection of circulating antigen or antibody may be useful in clinical diagnosis or monitoring of patients with these malignancies.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Collection of Serial Serum Samples from Cancer Patients

Principal Investigator: Dr. Carl Feit
Performing Organization: Memorial Hospital
City and State: New York, NY

Contract Number: NO1-CB-74124
Starting Date: 9/30/77 Expiration Date: 9/29/82

Goal: Collect serial serum specimens from cancer patients and benign disease controls for evaluation of potential immunodiagnostic tests for cancer.

Approach: Collect serial specimens from patients with melanoma, ovarian carcinoma, lung carcinoma, and Hodgkin's disease, plus suitably matched patients with benign skin lesions, benign adnexal masses, a history of heavy smoking, and benign lymphadenopathy to serve as controls.

Progress: This contract is completing the third year of a projected five-year project for collection of serial serum specimens from cancer and control patients for use in evaluating potential immunodiagnostic tests for cancer. Patient entry has been under way for a year and an average of 16 patients per month have been admitted to the study. Nearly 5,000 ampules of serum have been collected and stored, and extensive clinical information has been logged in. It is projected that entry of patients into the study should be completed by late 1980; then two additional years of follow-up specimen collection are required. A statistician has been consulted to determine whether an increase in frequency of sample collection or size would be desirable to get the most benefit from the contracted work.

Significance to Cancer Research: The effective evaluation of the prognostic value of immunodiagnostic tests requires testing sera from patients and controls which are well matched and have been collected in a serial prospective fashion. Establishing collections of such sera will expedite the evaluation of new immunodiagnostic assays as they become available.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $134,199

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer--Early Detection, Localization, and Therapy

Principal Investigator: Dr. Myron R. Melamed
Performing Organization: Memorial Hospital
City and State: New York, NY

Contract Number: NO1-CB-45007
Starting Date: 9/1/73 Expiration Date: Indefinite

Goal: To evaluate sputum cytopathology as a supplement to annual chest x-rays in detecting pulmonary neoplasms at an "early stage" and to evaluate efficacy of techniques for prompt localization of radiologically occult lung cancer (e.g., before progression to x-ray positive); in general, to evaluate efficacy of such screening to reduce lung cancer mortality.

Approach: Over a 3-year period, 5,000 test subjects and 5,000 control subjects will be entered into this study with active screening for 5 years, all to be followed for at least an additional 5-year period, for sufficient years to be statistically significant to answer pertinent questions. This will be conducted according to a protocol developed in conjunction with the Johns Hopkins University and the Mayo Foundation.

Progress: Recruitment for the Memorial Sloan-Kettering Cancer Center National Lung Program was completed in January 1978. Total enrollment is 10,040. Participants were randomly assigned to study group (A) (4,969), receiving annual chest x-rays and 4 monthly sputum; and to control group (B) (5,071), receiving annual x-rays only. There have been a total of 163 confirmed lung cancers identified so far, 91 in group A and 72 in group B. The principal mode of detection in the A group was by cytology in 19 cases, x-ray in 49 cases and both techniques in 7 cases. Of the 19 cases detected by cytology, 12 were Stage 0 or I; of the 49 cases detected by radiology (or radiology and cytology) in group A and the 52 cases detected by radiology in group B, a total of 101 cases, there were 51 in Stage I. There were 36 interval cancers diagnosed following symptoms or signs, and 17 were oat cell cancers. Among the 91 lung cancers appearing in group A, 33 were prevalence cancers for a prevalence rate of 6.6/1000 and 58 were incidence cancers for an incidence rate of 3.3/1000/year. In group B the 72 lung cancers included 21 prevalence for a prevalence rate of 4.1/1000 and 51 incidence cases, for an incidence rate of 2.9/1000/year. Survival rates so far have been excellent for the cases detected early (Stage 0, I), approximating better than 90%.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $640,485

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Methods of Screening for Endometrial Cancers by Uterine Samples

Principal Investigator: Dr. Leopold Koss
Performing Organization: Montefiore Hospital
City and State: Bronx, NY

Contract Number: N01-CB-84233
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: To carry out an extensive study to test the feasibility of screening for endometrial cancers in asymptomatic women by means of uterine sampling.

Approach: Study subjects will be selected from women over age 45 and up to age 70 who regularly attend the clinic of the contractor's institution. From the group, 3,200 women will be enrolled into the study. Sixteen hundred of the women will be in the age range 46 to 59 years and 1,600 will be in the age range of 60 to 70 years. After history has been obtained and the examining procedure explained, each consenting examinee will receive a complete gynecologic examination, a vaginal pool smear, a cervical scrape smear, and an endocervical aspiration smear. Examinees with odd clinic numbers will receive a Mimark endometrial sampling, and the examinees with even clinic numbers will receive the Isaacs (Curity) endometrial sampling. The patient's acceptance of the procedure will be evaluated and recorded by the gynecologist upon completion of the examination. Results will be communicated to the patient's primary physician and to the patient.

Progress: After a 3-month period of hiring and organization, the enrollment began in January 1979. As of November 6, 1979, 1,256 patients were screened. Data on the first 899 examinees, from January to August 1979, were evaluated by computer. White patients accounted for 82.8% of the population; black patients, 10.35%; and patients of Hispanic origin, 2.89%. About 19% of the patients were below age 50, 47% were 50-59 years of age, and the remainder, 60 years of age or over. Approximately 20% of patients were hormone users. Less than 2% of patients experienced severe discomfort during endometrial sampling and very few complained of pain upon completion of the procedure. Endometrial sampling was successfully accomplished in 92% of the examinees; in 4.2% the sampling could not be done and the results were debatable in 3.7%. In many patients of the last category there was sufficient material for laboratory evaluation. Among the first 1,000 examinees, 7 patients with histologically documented endometrial carcinoma were observed as well as a small group of patients with various forms of endometrial hyperplasia. In addition, 5 precancerous lesions of the uterine cervix, 1 carcinoma of the ovary, 1 carcinoma in situ of the vulva, and 3 carcinomas of the breast were discovered.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $193,440

CONTRACT RESEARCH SUMMARY

Title: Cryopreservation of Human Monocytes

Principal Investigator: Dr. J. A. Loos
Performing Organization: Netherlands Red Cross
City and State: Amsterdam, The Netherlands

Contract Number: NO1-CB-74164
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Find the optimal conditions for cryopreservation of human monocytes.

Approach: Analyze the following variables: purification methods, concentration of cryoprotectant, controlled cooling rate, the thawing procedure, and dilution and subsequent removal of the cryoprotectant. Once optimal cryopreservation is achieved, functional studies will be done.

Progress: Work on cryopreservation of monocytes was continued with the adherence method, which has a high yield but also a high percentage of dead cells, and the centrifuge method with a lower yield but very low percentage of dead cells. Improvement of the centrifuge method resulted in the same yield (70%) and the same purity (80%) as obtained with the adherence method. The following parameters were tested before and after freezing and thawing: recovery, oxygen consumption during phagocytosis, cytotoxicity against anti-D-sensitized red cells, chemotaxis toward casein, the capacity to phagocytose S. aureus, and helper function in mitogenic response to Con A. The following freezing conditions were varied: cell medium composition, concentration of cryoprotectant (DMSO), cell concentration, ampule volume, and cooling rate. The contractor's studies have led to the conclusion that human monocytes can be frozen for functional preservation. Macrophages isolated by buoyant density centrifugation from ascitic fluid of cancer patients showed similar functional properties before and after freezing and thawing under the standard conditions for monocytes.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Study of Innovative Techniques to Facilitate Passage of Colonoscope to the Cecum

Principal Investigator: Dr. Max Epstein
Performing Organization: Northwestern University
City and State: Evanston, IL

Contract Number: N01-CB-74114
Starting Date: 9/26/77 Expiration Date: 9/25/81

Goal: To develop safe and simple techniques and devices for early detection of small cancer lesions by means of endoscopic examination of the entire colon in order to improve life expectancy of postoperative patients and prevent or treat colonic cancer by endoscopic polypectomy.

Approach: Several devices will be investigated to enhance safety and ease of advancement of currently available colonoscope to the cecum. Prototype design, fabrication, and preliminary tests in simulated environment models during the first year will be followed by animal tests during the second year. During the second year, the most promising devices will be fabricated in an appropriate form for clinical testing. During the third year the main efforts will be directed at clinical trials.

Progress: Development of four devices has been carried to various stages of investigation, including tests on humans. 1) The endoscopic extender is a miniature endoscope that can be introduced through the ancillary channel of the conventional colonoscope to explore beyond obstructions and acute angulations of the lumen. It is 2.1 m long, has a diameter of 3.15 mm, and contains imaging, illumination, as well as lumens for passage of air and water and guiding of its own which makes it a first of its kind. Results of tests using this device, which included visualization of the small bowel through the ileocecal valve, were reported at the Annual Meeting of the American Society for Gastrointestinal Endoscopy on May 23, 1979, and were also submitted for publication in the Journal on Gastrointestinal Endoscopy. 2) The inflatable liner has been tested in dogs and in humans. A new version of a retractable inflatable liner appears to hold promise in development of an accessory for passing the colonoscope to the cecum. 3) A preliminary version of an overtube was constructed which should facilitate safe passage of the colonoscope particularly in cases of repeated insertion and withdrawal as in multiple polypectomies. 4) Crawling sleeve is intended to guide and pull the colonoscope at its tip, by means of a multiplicity of elastic inverted protrusions which, when activated by gas or liquid pressure, produce a hysteretic movement. The design of the appropriate mold to produce the device has been completed and prototypes will be fabricated and tested.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $19,436

CONTRACT RESEARCH SUMMARY

Title: Clinical Application of Assays for Tumor-associated Antigens

Principal Investigator: Dr. Clement C. S. Hsu
Performing Organization: Northwestern University
City and State: Evanston, IL

Contract Number: NO1-CB-74132
Starting Date: 9/26/77 Expiration Date: 9/25/80

Goal: To investigate the clinical applicability of rabbit antisera, anti-EP and anti-ALL, prepared in our laboratory, and the clinical relevance of cell-bound blocking component on leukemic lymphocytes.

Approach: By serial examination of lymphocytes from patients with acute lymphocytic leukemias (ALL) during the course of illness and from patients with other diseases for the anti-EP and anti-ALL reactivity.

Progress: ALL cell-associated antigens (ALLA) negative cases have a poorer prognosis than ALLA positive cases. Some ALL patients with no lymphoblasts in blood may have up to 49% ALLA carrying cells in circulation. ALLA positivity may be lost during the relapse of the disease. An Fc receptor pre-empting technique has been developed and utilized routinely to eliminate nonspecific immunofluorescent staining of the cell membrane. Other rabbit antisera to Ia and to ALLA have been prepared by immunizing rabbits with an established B-cell line and ALL cell line. Circulating immune complexes in ALL plasmas have been quantitated employing 3.5% polyethylene glycol precipitation. Antigen enhancement by overnight cell incubation can be documented in approximately 1/3 of ALL cases. An "antibody pre-labeling" technique has been developed which allows detection of unequivocally endogenous cell surface molecules, and in vitro investigation of factors affecting the membrane antigen expression. An immunofluorescent immune complex adsorption (IFICA) assay is being devised which allows detection of less than 1.0 μg/ml of a soluble antigen. The assay may enable the contractor to test for the presence of minute quantities of ALLA or Ia in ALL serum.

Significance to Cancer Research: Our results indicate the usefulness of anti-ALL in differential diagnosis and subclassification of ALL. Further studies of cell-bound blocking component may lead to understanding of another mechanism by which leukemic cells evade recognition and destruction by host immunological apparatus.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Contrast Agents to Improve Detection of Small Liver Metastases with Computerized X-Ray Tomography

Principal Investigator:	Dr. Michael A. Davis
Performing Organization:	Peter Bent Brigham Hospital
City and State:	Boston, MA

Contract Number:	N01-CB-84234		
Starting Date:	7/1/78	Expiration Date:	6/30/81

Goal: To improve detection of small metastatic lesions in the liver by improved use of contrast agents to provide appropriate image enhancement in x-ray computerized tomographic studies.

Approach: The work, which will be carried out over a three-year period, will consist of six tasks: synthesis of new hepatic-specific contrast agents; preliminary evaluation in animal models; toxicity evaluation in animals; pilot clinical studies with currently available contrast agents; pilot clinical studies of promising new agents with acceptable toxicity characteristics; and clinical trials on the most promising contrast agent.

Progress: A computer model, confirmed by phantom studies, revealed that increasing background radiopacity enhances detectability of a non-opaque target lesion in CT. This model is analogous to small metastatic nodules in normal hepatic parenchyma. Further computer analysis, also confirmed by phantom studies, indicated that elements close to atomic number 60 displayed the greatest absorption at kilovoltages commonly used in CT. Therefore suspensions of cerium, dysprosium, gadolinium oxides, and silver iodide were prepared and evaluated in a rabbit liver V2 carcinoma model. Preliminary toxicity studies were also performed. Density of hepatic parenchyma was increased appreciably with only minimal elevation of tumor density, thus enhancing considerably detectability of V2 carcinoma metastases when compared with studies using standard intravenous iodinated contrast agents in similar doses. Lesions 4 mm or larger were easily detected.

A second line of investigation has focused on iodinated analogs of the new hepatobiliary agent HIDA. The fact that changes in the water-lipid partition coefficient dramatically affect hepatic extraction of these agents is being utilized in the design of additional derivatives. Human studies with standard i.v. contrast agents indicate that all primary tumors and metastases thus far encountered display contrast transit characteristics which differ from normal hepatic parenchyma. Thus, rapid scanning reveals data useful in differentiating tumors from normal liver, cysts, or abscesses.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera from Populations with High Cancer Risk

Principal Investigator: Dr. Henry Altschuler
Performing Organization: Philadelphia Geriatric Center
City and State: Philadelphia, PA

Contract Number: NO1-CB-33914
Starting Date: 6/25/73 Expiration Date: 10/24/80

Goal: To collect serial serum specimens from populations at high risk of contracting malignant disease for the purpose of developing and evaluating immunodiagnostic screening tests for human cancer.

Approach: Demographically stable populations at high risk of developing human malignancies in the colon, lung, breast, prostate, bladder, pancreas, etc. will be followed on an annual basis. This includes clinical evaluation and serum collection. The sera will be banked and records kept on the patients so that information obtained from the stored sera can be correlated with development of any particular cancer.

Progress: Over the past 6 years and 3 months, a total of 4,203 serum specimens (divided into ten 1-ml aliquots) have been collected, processed, logged with assigned numbers on a chronological basis, frozen, and shipped frozen in dry ice to the Regional Serum Storage Center at the Mayo Clinic in Rochester, Minnesota. In addition, there is a patient card index, alphabetically organized by patient's name, and including age, sex, hospital MDI number, address, diagnoses, serum numbers (a label for each date of collection of blood placed on card), and date of collection of blood. There are additional cards which contain such information as the date of the last physical examination and information concerning such conditions as asthma, allergy, rheumatoid arthritis, desensitization therapy, immunosuppressive therapy, immunization (influenza vaccine), and blood transfusions. Also maintained are a cancer index file and a death index card file with significant findings at autopsy if performed. Data from these files are being collated on special computer adaptable forms supplied by NCI. An overall detailed clinical summary is being completed.

Significance to Cancer Research: Early detection of cancer by means of appropriate testing would permit treatment of cancer at the most favorable time.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $68,153

CONTRACT RESEARCH SUMMARY

Title: Application of Digital Image Processing Techniques to Cytology Automation

Principal Investigator: Dr. James W. Bacus
Performing Organization: Rush-Presbyterian-St. Luke's Medical Center
City and State: Chicago, IL

Contract Number: N01-CB-74190
Starting Date: 9/30/77 Expiration Date: 9/29/81

Goal: Development of optimal algorithms for cell classification applicable to automated digital image processing system.

Approach: The contractor shall conduct a comprehensive study to determine single cell vs. specimen classification accuracies for image processing algorithms and for cytotechnologist. This will determine how well the machine algorithms are performing. Five experimental tasks will be done: (1) sample acquisition, (2) cell acquisition, (3) observer recognition, (4) cell classification algorithms development, and (5) analysis and evaluation of results.

Progress: Patient selection and sample acquisition are close to completion. Fabrication of the cell acquisition system is essentially finished. Software for the various aspects of the cell acquisition protocol is finished. Cells are being digitized and photographed routinely with this equipment. Programming of cell recognition algorithms continues with emphasis on scene segmentation technique and cell classification. Work has begun to design specific statistical experimental protocol for the observer recognition experiment.

Project Officer: Dr. Bill Bunnag
Program: Diagnosis Branch
Technical Review Group: Cytology Automation Committee
FY 80 Funds: $401,580

CONTRACT RESEARCH SUMMARY

Title: Isolation and Tissue Culture of Human Tumor Cells

Principal Investigator: Dr. Jorgen E. Fogh
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-43854
Starting Date: 8/8/73 Expiration Date: 8/7/81

Goal: To develop further procedures for encouraging tumor cell growth in culture and to perform tests necessary to define standard cell lines from various tumors.

Approach: Human tumor cell lines are established in culture from surgical specimens and are collected from other investigators. They are characterized in this laboratory as to malignancy, cell type, and special features as needed for individual investigations.

Progress: The contract supports a resource in the form of an extensive bank of human tumor lines grown in vitro. Cell lines are preserved after as few passages as possible, and are characterized in terms of morphology, nutritional requirements, growth in vitro, chromosomal characteristics and poliovirus susceptibility. They are monitored for microbial contamination. Analysis of polymorphic enzyme patterns enable the investigators to determine possible cross-contamination with other cell lines and to exclude such contamination when unique phenotype combinations are observed. The 426 cultured human tumor cell lines are available to other investigators. The bank now includes 69 melanomas, 31 carcinomas of the breast, 20 brain and nervous tissue tumors, 19 carcinomas of the bladder, 18 of the colon, 17 of the lung, and 10 of the kidney. Five hundred twenty lines have been made available to other investigators in a 12-month period (1979), indicating a good utilization of the resource.

Significance to Cancer Research: The collection of well-characterized contamination-free human tumor cell lines is pertinent for many disciplines of experimental and clinical cancer research.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $134,757

CONTRACT RESEARCH SUMMARY

Title: In Vitro Augmentation of Cell-mediated Cytotoxicity

Principal Investigator: Dr. Philip Livingston
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-74130
Starting Date: 9/30/77 Expiration Date: 9/20/80

Goal: To develop means to augment cell-mediated cytotoxicity (CMC).

Approach: Exposure of melanoma patient lymphocytes to autologous melanoma cells in vitro under various experimental conditions; determination of the specificity of antigens detected in cytotoxicity tests by cellular immunoadsorption and inhibition tests with fixed and unfixed tissue; and characterization of melanoma-specific effector cells as opposed to natural killer cells and suppressor cells.

Progress: Tests with fresh peripheral blood lymphocytes (PBL) on autologous melanoma cells demonstrated consistent CMC in 18 of 32 patients. Limitations imposed by the small number of fresh effector cells available and by fluctuations in their reactivity, however, frustrated attempts at adopting cold target cell inhibition or cellular immunoadsorption tests for specificity analysis in this system. CMC was not detected following incubation of PBL with autologous melanoma cells for 5-8 days. When TCGF was added following the sensitization procedure, however, continuous (TCGF dependent) cell lines have resulted. These cells have a tripling time of 3-6 days, retain their initial pattern of reactivity as measured in the 6-hr ^{51}Cr release assay and show significantly stronger reactivity than fresh peripheral blood lymphocytes or lymphocytes tested immediately after in vitro sensitization but before culture in TCGF. One of these lines has now been in continuous culture for more than 4 months. Cloning of some of their T cell lines has been started. Early results indicate that different clones show different reactivity and suggest that propagation of clones with strong specific reactivity (one clone shows 60% CMC at an effector/target cell ratio of 1/1) can provide superior reagents for the study of melanoma cell surface antigens recognized by cytotoxic T cells.

Significance to Cancer Research: We expect this study to contribute to our understanding of cellular immunological rejection mechanisms, and to the development of methods for strengthening these mechanisms.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Serologic Assays with Purified Melanoma-associated Antigens

Principal Investigator: Dr. Kenneth O. Lloyd
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-74127
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Develop an assay for new tumor-associated antigens.

Approach: Isolate and characterize cell surface antigens of human malignant melanoma and develop a sensitive serologic assay for monitoring and diagnosis of melanoma.

Progress: Heterologous anti-melanoma sera have been prepared against antigens originally defined using serological assays in autologous melanoma systems. By these assays it has been shown that human melanoma possesses melanoma specific antigens. Antisera to three different antigens have been prepared. One antiserum is against whole melanoma cells, and recognizes a common antigen shared between melanoma and other tumor cells. A second antiserum is prepared against a "shed" glycoprotein sample known to contain a melanoma antigen detected by the autologous typing approach. After absorptions with lymphocytes and non-melanoma tumor cell lines, this antiserum has a low titer of antibody directed against melanoma cells. The third antiserum is prepared against a cell surface glycoprotein that binds both Con A and WGA lectins. Although this antigen (molecular weight: 100,000) has a widespread distribution, it may show variability in its carbohydrate structure in cancer cells; this latter property may be useful as a marker for malignancy. More recently, monoclonal antibodies have been prepared against melanoma cells; the specificity of these reagents is currently being examined.

Significance to Cancer Research: New sensitive assays are needed for the immunodiagnosis and monitoring of malignant melanomas.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: HLA Typing of Human Tissue Culture Cell Lines

Principal Investigator: Dr. Marilyn Pollack
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-84255
Starting Date: 9/30/78 Expiration Date: 2/1/80

Goal: Determine the HLA antigens present on some frequently used human tissue culture cell lines derived from tumors and relate them to the HLA phenotype of lymphocytes from the cell line donor, where possible.

Approach: Perform HLA typing using a modification of the complement-dependent microcytotoxicity procedure. The HLA-A, -B and -C locus antigens expressed on at least 45 frequently used human tissue culture cell lines will be identified and DR alloantigens will be screened for.

Progress: Forty-six frequently used or patient/donor available human tissue culture lines were HLA typed using a modification of the complement-dependent microcytotoxicity procedure in which complement and incubation conditions suitable for each cell line were carefully selected. In cases where more than two HLA antigens corresponding to a single HLA locus were detected by direct cytotoxicity tests, specificity of the antigens present was verified by absorption/inhibition of specific typing sera which were subsequently tested for residual activity against a panel of antigen positive lymphocytes, in parallel with unabsorbed sera. A new 2-color fluorescence cytotoxicity test was also used to verify antigen assignments in some other cases. Twenty-three of the cell lines tested did not have donors available for comparative typing. The remaining cell lines were tested in parallel with peripheral lymphocytes from their available donors, or typing results were compared with those obtained previously from donors who were no longer available. One of two of the bladder carcinoma cell lines tested and more than half of the twenty-four melanoma cell lines tested were found to express DR alloantigens in addition to HLA-A, B and C antigens. The presence of these antigens was verified by immunofluorescence and absorption/inhibition typing. In all cases where donor-B cells were available for comparative testing, the allospecificity of the tumor cell line DR antigens was the same as the donors'.

Significance to Cancer Research: Identification of HLA antigens on the surface of human tumor tissue culture cells may be useful in the identification and characterization of tumor immunodiagnosis associated antigens on such cells.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Development of Ultrasonic Probes To Be Inserted Through Endoscopes for Use in Cancer Diagnosis

Principal Investigator: Mr. James L. Buxton
Performing Organization: SRI International
City and State: Menlo Park, CA

Contract Number: N01-CB-74136
Starting Date: 9/30/77 Expiration Date: 1/29/81

Goal: To develop and clinically test an ultrasonic imaging system which will provide extremely high resolution images of the anatomy in the thorax and upper abdomen.

Approach: Currently available imaging systems have inadequate resolution to diagnose many diseases in organs in the middle of the torso. For instance, conventional imaging systems cannot diagnose pancreatic cancer until very advanced stages. The thrust of this project is to develop an ultrasonic imaging system with its transducer on the tip of an endoscope, so that internal organs can be imaged through the wall of the gastrointestinal tract. The resulting proximity to internal organs permits high resolution images, and, in addition, eliminates the shadowing by bowel gas which plagues conventional ultrasonic imaging systems.

Progress: By the end of the first year, the basic ultrasonic imaging system had been developed and demonstrated. The system is organized around a 64 element, 10 MHz, linear array which is 30 mm long. The 3 x 4 cm image is produced in a B-scan format at approximately 30 frames per second. Dynamic focusing on both transmit and receive provides submillimeter resolution in both dimensions throughout the field of view. Midway through the second year of the project, a prototype endoscopic imaging system was installed at the Mayo Clinic for tests with dogs. This first system included an endoscope with an outside diameter of 13 mm, side-looking optics and ultrasound, and a rigid tip length of 80 mm. This system demonstrated that intragastric imaging provides very high resolution imaging of the anatomy near the upper GI tract. Among the organs imaged were: heart, liver, both kidneys, gall bladder and biliary tree, spleen, and abdominal vasculature. Early in the third year of the project, an improved endoscope was completed. Changing the optics to look forward allowed the rigid tip length to be reduced to 40 mm. With this unit, clinical trials were started in November 1979. In humans, this system has imaged all of the organs that the first system imaged in dogs, and in addition, has demonstrated the entire pancreas.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Direct Assay for Lymphokine

Principal Investigator: Dr. Zoltan Lucas
Performing Organization: Stanford University
City and State: Stanford, CA

Contract Number: NO1-CB-84263
Starting Date: 9/30/78 Expiration Date: 9/29/80

Goal: Development of more rapid and quantitative methods than currently available for assaying lymphokines.

Approach: Prepare reagents and develop sensitive radioimmunoassays for α-LT, β-LT, and IDS from human lymphocytes.

Progress: The contractor has shown that α-LT is a 76,000 dalton glycoprotein that is not composed of subunits and that previously ascribed associations of LT with the 45,000 dalton, extracellular β-LT, are fortuitous. This was accomplished by electrophoresing highly purified 76,000 dalton α-LT in SDS-polyacrylamide gel under reducing conditions, eluting from gel segments, removing SDS by column chromatography on AG11A8 ion retardation resin and determining that all recovered activity was in the 76,000 dalton fraction. The most highly purified preparations of α-LT (2000 to 3000-fold purified) still contain protein impurities, perhaps as much as 90-95% of mass of the preparation. The impurity is 76,000 daltons and is more readily labeled with ^{125}I than α-LT. The impurity, made by human tonsil cells never in contact with FCS, reacts with antibody to FCS. The impurity was not detected in adult human serum. In preparations of α-LT from cultured lymphocytes containing FCS, this contaminating protein is 76,000 daltons (SDS-PAGE); from cultures without FCS, it occurs as two smaller strands, about 50,000 and 22,000 daltons. This indicates the presence of an active protease in lymphocyte cultures which may explain both the marked charge heterogeneity of α-LT and the size heterogeneity of α-LT described by other workers.

Significance to Cancer Research: Improvement of methods for cancer detection, diagnosis, and screening.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Antibodies of Syngeneic Mouse Tumors

Principal Investigator: Dr. Isaac P. Witz
Performing Organization: Tel Aviv University
City and State: Tel Aviv, Israel

Contract Number: NO1-CB-74134
Starting Date: 9/30/77 Expiration Date: 9/29/81

Goal: Study alterations in the serologic reactivity pattern in mice at high risk of developing carcinogen-induced or endogenous virus-induced malignancies as well as reactivity of such mice toward antigens associated with the relevant tumors.

Approach: Use the following animal model systems: spontaneous tumors in C3HeB mice; mammary tumors induced by DMBA in BALB/c mice; and lung adenomas in urethan-treated BALB/c mice.

Progress: This contract has completed its second year of work, studying alterations of serological and cellular reactivity pattern in mice at high risk of developing malignancies. Breeding colonies of the appropriate mice have been set up for these longitudinal studies. The serologic pattern of normal mice has been established to serve as a basis for comparison of altered reactivity. Various technical and methodological aspects have been analyzed to understand what effects bleeding, aging, and unrelated immunizations may have on the anti-tumor serologic reactivity of these mice. These baseline studies will enable the contractor to do the analyses of the altered serologic reactivities as carcinogen and viral-induced malignancies develop. It has been established that urethan treatment, or bearing of primary lung adenomas induced by urethan, has no measurable effects on the following naturally occurring reactivities: (1) antibody-mediated complement-dependent lysis, (2) NK activity of splenocytes, and (3) cytostasis mediated by splenocytes. DMBA treatment of BALB/c mice caused a severe but transient depression in the ability of splenocytes from the treated mice to mediate NK activity and cytostasis of adherent target tumor cells. Both activities were restored to normal levels before the development of primary tumors. BALB/c mice bearing DMBA-induced primary tumors show the following patterns: (1) depressed natural antibody activity, (2) depressed NK activity, and (3) elevated cytostatic activity. C3HeB mice bearing spontaneous carcinomas have a depressed NK activity.

Significance to Cancer Research: The results will indicate whether or not altered serologic activity against tumor-associated antigens signals the appearance of primary or transplanted tumors.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $96,800

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Ovarian Carcinoma in Asymptomatic Women

Principal Investigator: Dr. Leo Stolbach
Performing Organization: Tufts University Pondville Hospital
City and State: Norfolk, MA

Contract Number: N01-CB-64028
Starting Date: 6/30/76 Expiration Date: 6/30/80

Goal: To develop a test for ovarian carcinoma which would be useful in the diagnosis of cancer of the ovary and in following the course of the disease during treatment.

Approach: Malignant effusion fluids of patients with ovarian carcinoma will be processed in order to (1) isolate and purify tumor-specific antibodies and/or antibody-antigen complexes from effusion fluids; (2) dissociate the antibody-antigen complexes and separate the tumor-associated antigens and antibodies making up these complexes; (3) determine the immune specificity of antibodies isolated from the effusion fluids; (4) detect tumor-associated antigen in the effusion fluid and tumor extracts; (5) utilize the tumor-specific antibodies to prepare an immunoadsorbent for the purification of the antigen; and (6) develop a diagnostic assay for detecting circulating antibody, antigen, and antigen-antibody complexes in patients with various stages of ovarian malignancy.

Progress: Immune complexes have been isolated from malignant effusion fluids. The IgG from these complexes binds specifically to ovarian cancer cells, as shown by indirect immunofluorescence studies carried out on sections of ovarian cancer tissue. Ovarian cancer antigen can also be purified from these complexes and has been used for the production of xenoantisera in rabbits. These xenoantisera, after absorption with normal human plasma and extract of normal ovary, can identify fluids from patients with cancer of the ovary in assays utilizing laser nephelometry or complement fixation. These xenoantisera, however, do not react to a significant extent with sera or plasma from these patients. Testing of the xenoantisera is currently underway for their use in a more sensitive enzyme-linked immunosorbent assay (ELISA). This assay will be used to measure the level of ovarian cancer associated antigen, antibody, and antigen-containing immune complexes in serum, fluid and tissue samples.

Project Officer: Mr. Louis P. Greenberg
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $9,885

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Applications of Human Tumor-associated Antigens

Principal Investigator: Dr. Ilkka P. T. Hakkinen
Performing Organization: Turku University
City and State: Turku, Finland

Contract Number: NO1-CB-64070
Starting Date: 9/30/76 Expiration Date: 6/29/80

Goal: To evaluate the FS antigen (fetal sulfoglycoprotein antigen) in screening for early gastric cancer. To characterize the FS antigen and standardize the reaction, a trial mass-screening for gastric cancer is being carried out among an unselected population. Clinical material is used to test the FSA method in discriminating between benign and malignant gastric ulceration.

Approach: Acid glycoproteins from gastric juice are tested by immunodiffusion against FS-antiserum, its preparation being standardized as far as possible. Subjects analyzed as secreting FSA are examined for gastric alterations by gastroscopy, and the entire cohort followed for two years. Cancer rates will be compared with actual statistics.

Progress: More than 95% of clinical gastric cancers were earlier found to secrete FSA in gastric juice. Results of this mass-screening trial on 53,020 subjects seem to support the hypothesis that FSA secretors form a high-risk group for gastric cancer; after two years the majority of cancers were found in the small minority (8.8%) of FSA-positive subjects, and after three years the rate still was over 50% (preliminary result). Figures for prognosis are not yet available, but there were a great many early cancers (over 40%) among the gastric cancers found in FSA secretors. The final purification procedure of FSA (starting from cancerous gastric juice) has been developed. The chemical composition and molecular size of purified FSA have been determined.

Significance to Cancer Research: If the method is proved to be useful, it will provide a new tool for early diagnosis of gastric cancer.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Radioisotope Surface Markers and Detectors for Endoscopic Techniques

Principal Investigator: Dr. James M. Woolfenden
Performing Organization: University of Arizona
City and State: Tucson, AZ

Contract Number: N01-CB-64012
Starting Date: 6/30/76 Expiration Date: 12/29/80

Goal: To establish a means for improving early detection of small cancer lesions on endoscopically approachable surfaces in order to improve the life expectancy of the patient.

Approach: A system will be developed for endoscopic detection of small cancer lesions in mucosal surfaces using tumor-seeking radioisotopic markers. A miniature radiation detector system suitably matched to emission characteristics of the isotopic markers will be developed. During the first year, work will center on development and testing of tumor-seeking markers. Initial detector design and construction will begin. During the second year, development of markers will continue, and candidate detector systems will be tested using a tumor phantom system. The period from the beginning of the third year to the termination of the contract will concentrate on patient studies to assess the sensitivity, specificity and accuracy of the tumor markers and detector system in localizing early cancer lesions.

Progress: Scintillation detectors made of thallium-activated sodium iodide and thallium-activated cesium iodide and a cadmium telluride semiconductor detector have been constructed. All have performed satisfactorily in tumor phantom studies, with better performance by the NaI(Tl) and CdTe detectors than the CsI(Tl). The NaI(Tl) detector is currently being used in clinical patient studies. The tumor-seeking marker is cobalt-57 bleomycin. Standard external imaging is performed 24 hours after intravenous injection of 1 mCi Co-57 bleomycin; standard fiberoptic bronchoscopy is then done. The detector, coupled to an external photomultiplier tube by a flexible fiberoptic light guide, is passed through the bronchoscope channel, and counts are taken at standard sites throughout the bronchial tree. Preliminary results indicate that detector sensitivity for tumor detection is similar to that of bronchoscopy and chest radiography, and that bronchoscopically invisible submucosal tumors and occult sources of malignant cytology can be found using the detector system in some patients. Further studies are in progress to assess minimum detectable tumor size and to define further the sensitivity and specificity of the technique. Further studies are in progress in animal tumor models to assess whether enhanced tumor uptake of the radiolabeled marker can be achieved using individual peptide fractions or analogues of bleomycin.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cell-mediated Reactivity of Normal Persons to Human Tumor-associated Antigens

Principal Investigator: Dr. Mitsuo Takasugi
Performing Organization: University of California
City and State: Los Angeles, CA

Contract Number: NO1-CB-74133
Starting Date: 9/16/77 Expiration Date: 9/15/80

Goal: Investigate the significance of the fact that effector cells from healthy individuals and cancer patients are normally cytotoxic to cultured human tumor cells.

Approach: Examine the level and specificity of the reaction with effector cells isolated from healthy people and patients; study the level of cells bearing Fc receptors in the circulation and how these levels are affected by cancer.

Progress: Studies are progressing in the identification of some specificities detected by natural cell-mediated cytotoxicity (NCMC) toward understanding the function of Fc receptors on effector cells, and the control of NCMC with regulatory lymphokines. Selective NCMC has been detected against EBV- and HSV-associated antigens, HLA and natural target antigens. The similarity of NCMC with antibody-dependent cell-mediated cytotoxicity (ADCC) has been demonstrated; both tests employ heterogeneous populations of effector cells but share at least one subset of cells with Fc receptors. Antibodies arm effector cells first in NCMC, and ADCC is controlled by the production of interferon and a factor which suppresses NCMC (N-cell inhibitory factor or NIF). Interferon is induced by culturing lymphocytes with allogeneic cells, poly I-C, viruses and other stimulators. NIF is produced by unstimulated lymphocytes. NCMC is a specific phenomenon directed by antibodies and controlled by two opposing regulatory lymphokines.

Significance to Cancer Research: It appears that natural cell-mediated cytotoxicity is a cell-mediated cytotoxic system whose specificities are directed by antibodies in the circulation. A better understanding of these processes and how cancer affects them is of great significance.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Imaging Capability Improvement and Evaluation in Detecting Early Pancreatic Cancer

Principal Investigator: Dr. A. R. Moossa
Performing Organization: University of Chicago
City and State: Chicago, IL

Contract Number: NO1-CB-84232
Starting Date: 8/1/78 Expiration Date: 7/31/81

Goal: To compare, improve, and evaluate imaging methods and strategies applied to early detection and diagnosis of pancreatic cancer.

Approach: The imaging methods and strategies will be applied to the study of a selected population of patients in whom there is a high clinical probability of pancreatic cancer. The group will include patients with pathological conditions not related to the pancreas, patients with benign pancreatic disease, and patients with pancreatic cancer. The purpose of this study is to improve the ability of the diagnostic team to achieve rapid and certain identification of patients with early pancreatic cancer within the high probability group of patients. Patients must be over 35 years of age and in suitable physical condition to tolerate the necessary investigations and major abdominal surgery. They must have an estimated survival of at least 3 years. Both sexes will be included except for exclusion of pregnant women. The study will be organized into five separate projects. All of the various imaging methods to be investigated will be included in one of four projects. The fifth project will be concerned with collection and evaluation of data gathered by the other four projects.

Progress: During the initial 14 months of the contract, 52 patients with clinical indications for probable pancreatic cancer were entered into this study; 31 proved to have pancreatic cancer, 15 pancreatitis and 6 had a normal pancreas. The combination of ultrasonography, endoscopic retrograde cholangiopancreatography (ERCP), computed tomography, and arteriography continues to show promising results. Radionuclide scans with ^{11}C-tryptophan are better than with ^{75}Se-selenomethionine produced by the local cyclotron and scanned with a special positron camera are now being supplemented by scanning with the Phocon (longitudinal multiplane emission tomography which was originally performed following selenomethionine injection). Radionuclide scans are now performed both in fasting and in stimulated (after a meal; after injection of CCK-PZ or urocholine) state. Development of computer programs to produce a synthesized composite image by superimposing multiple image continues. The technical difficulties of ERCP scanning following retrograde injection of technetium microspheres and of computerized ultrasound have now been sorted out. These approaches will be continued.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $242,272

CONTRACT RESEARCH SUMMARY

Title: Image Processing for Development of Automated Cell Recognition System

Principal Investigator: Dr. George L. Wied
Performing Organization: University of Chicago
City and State: Chicago, IL

Contract Number: NO1-CB-33873
Starting Date: 5/31/73 Expiration Date: 5/31/81

Goal: Automation of clinical cytologic screening and diagnosis, focusing on definition of information requirements and criteria for high throughput, high resolution processing-based decision making.

Approach: The contractor shall evaluate information requirements for characterization and recognition of normal, premalignant, and malignant cells from the human female genital tract by means of digital image processing.

Progress: Digitized cell images have been compiled. These data are stored on tapes and will be made available to other contractors for further analyses. These cells are from the various cell types encountered in the exfoliated cell specimens from the female genital tract. Image editing system is operational. Sorted cleaned cell images are available for other contractors to analyze with their own algorithms. Further details on scanned cells are being done using 3-color scanner. High-resolution data acquisition module and cell detection module hardware are being implemented. An integrated system is being tested using the best and optimal algorithms to determine the classification performance of the system to classify cells as normal and non-normal.

The hardware system was amplified by an array processor which improved data admission and evaluation speed significantly. The Cell Detect Module now uses the green channel videosignal of the 3-color camera, thus scanning the specimen with the same video system in the detect mode as well as in the actual diagnostic mode.

A fully integrated screening and diagnostic system consisting of a cell preparation system (Monolayer Device), a prescreening module (Cell Detect Module), and a diagnostic analysis module will be ready by 1981. It is also feasible to develop a system which terminates with the Cell Detect Module in an abbreviated system that would result in an instrument which scans the specimen automatically, and recalls the coordinates of those areas tagged as "alarms" which then may be re-assessed by human evaluators.

Project Officer: Dr. Bill Bunnag
Program: Diagnosis Branch
Technical Review Group: Cytology Automation Committee
FY 80 Funds: $270,155

CONTRACT RESEARCH SUMMARY

Title: Statistical Center for Cooperative Lung Cancer Groups

Principal Investigator: Dr. C. Ralph Buncher
Performing Organization: University of Cincinnati
City and State: Cincinnati, OH

Contract Number: N01-CB-43868
Starting Date: 6/1/74 Expiration Date: Indefinite

Goal: To collect and analyze information from the three collaborating clinical centers in the lung cancer screening program (which is directed toward the detection of early lung cancer in high-risk patients) to ascertain whether this screening program will reduce mortality and morbidity.

Approach: Procedures have been established with each of the centers and agreement has been reached concerning the common data base for this study. Data are being routinely monitored by the Statistical Center, translated into a single computer data base in Cincinnati, and analyzed to provide collaborative information as well as comparative information. Reports have been made quarterly. The Statistical Center will continue to search the data from this study for important and statistically significant findings.

Progress: The Cincinnati Central Statistical Group (CSG) has established a common data base for this three clinical center study of screening to detect lung cancer at an early enough time to reduce mortality. Translation and reporting systems for each of the clinical centers have been created and analyses of collaborative results to date have been provided to the NCI and participating clinical centers. Intake of subjects has been completed so that adherence and compliance with the screening program is a current focus. Several thousand subjects have completed the screening program. Survival analysis efforts are being made by CSG to obtain meaningful results with respect to mortality differences in the screening groups at as early a time as feasible. Regular mortality Review Committee conferences are held to discuss the death certificate and best information causes of death for each man in the study who has died. Other central roles have been fulfilled by the CSG. In the first part of this contract, CSG was also responsible for a five-center study of computed tomography compared to radionuclide scanning and other methods of detection and diagnosis of brain tumors. Publications from that study have been issued and additional reports are in progress. Similarly, CSG has worked with three centers concerning a collaborative study of pancreatic cancer diagnostic procedures. A number of reports from this study have been published.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $197,861

CONTRACT RESEARCH SUMMARY

Title: Cryopreservation of Human Monocytes for Use in Immunologic Studies

Principal Investigator: Dr. Roy S. Weiner
Performing Organization: University of Florida
City and State: Gainesville, FL

Contract Number: N01-CB-74131
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To isolate human monocytes from peripheral blood and develop methods for their adequate cryopreservation.

Approach: Develop methods of collection and isolation of peripheral blood monocytes that would be applicable to normal subjects and to patients with cancer. Develop optimum techniques for cryopreservation and define the functional characteristics of cryopreserved cells. Evaluate changes in monocyte structure and function occurring in patients with cancer.

Progress: This contract is in its third year. The contract workscope calls for optimization of separation and cryopreservation techniques for monocytes and to do functional studies on these cells from cancer patients and normal donors. To date, the contractor has separated large numbers of monocytes from the peripheral blood using pheresis techniques. Up to 2×10^8 monocytes can be collected in 60 ml of buffy coat from peripheral blood using a standard Haemonetics Model 30 blood cell separator. Further, isolation techniques have been developed to obtain purified monocytes by elutriation centrifugation. Techniques of Sanderson et al. have been modified so that up to 1.5×10^9 mononuclear cells can be loaded into the elutriation chamber and up to 3×10^8 monocytes are obtained in greater than 90% purity, representing 70% yield from peripheral blood. Additionally, a population of monocytes representing 15% of the total blood monocytes has been separated; this is significantly smaller than the large monocyte population. These cells make up only 5 to 10% of cells in the elutriation fraction containing 85% of the lymphocytes. The small mononuclear cells have been purified by reversible adherence on microexudate conditioned tissue culture plates. Studies continue on the functional characterization and optimal cryopreservation procedures on both of these peripheral blood monocyte subsets.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Development of Contrast Agents for Use in Clinical Ultrasonic Diagnosis

Principal Investigator:	Dr. Nabil F. Maklad
Performing Organization:	University of Kansas Medical Center
City and State:	Kansas City, KS

Contract Number:	N01-CB-84236		
Starting Date:	6/19/78	Expiration Date:	6/18/81

Goal: To improve capability of ultrasound in the imaging of small tumors through the use of appropriate contrast agents.

Approach: Chemical compounds with appropriate physical properties to influence the ultrasonic signal will be identified and equipment will be assembled to test such compounds. Differential uptake of individual contrast compounds will be measured in animal tumor models. Dosage requirements for optimum ultrasonic visualization will be determined in animals and, later, in human subjects. Animal toxicity studies will be made with promising compounds. Clinical trials will be conducted using the most promising contrast agent after appropriate toxicity studies have been completed.

Progress: A number of compounds were selected and categorized ultrasonically by measuring the speed of sound, density and acoustic impedance in their solution as a function of concentration. Sodium citrate and calcium gluconate were chosen because of their ability to alter speed of sound and acoustic impedance and because of their known pharmacological effects. Toxicity studies were performed and the optimum combination of these two compounds was determined. Organ distribution studies indicate a concentration of these two compounds in the kidneys. The optimum combination of these two compounds was injected intravenously into anesthetized dogs. Enhancement of echoes of canine renal cortex was demonstrated. These experiments indicate that introduction of appropriate aqueous solutions produces changes in scattering and reflection properties of tissue.

Solid microspheres were prepared, using various compounds including gelatin, collodion and collagen. Ultrasonic backscatter from these solid microspheres in suspension and from a standard erythrocyte suspension were measured. When normalized to a standard particle size and concentration, the backscatter from collagen was about 30 dB above erythrocytes. The collagen microspheres were chosen for preliminary in vivo canine experiments. As with other particles below 5 microns in diameter injected into the body, the collagen microspheres are taken up by RES cells, especially in liver and spleen. In vivo canine experiments using commercial diagnostic equipment (real time linear array at 7 MHz) demonstrated enhanced backscatter from liver after intravenous injection of the microspheres.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Purification of Human Tumor-associated Antigens

Principal Investigator: Dr. David Gold
Performing Organization: University of Kentucky
City and State: Lexington, KY

Contract Number: NO1-CB-84259
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: Purification, isolation, and identification of tumor-associated antigens.

Approach: Extract and purify colonic mucoprotein antigen (CMA) from normal colon and colonic adenocarcinomas; develop a radioimmunoassay and immunohistochemical procedures for detection of CMA; investigate the potential of CMA as a tissue or tumor-specific marker.

Progress: This contract is concerned with the isolation of colonic mucoprotein antigen (CMA) and development of a radioimmunoassay for detection of this organ-specific antigen. During the first year emphasis was placed on purification of the antigen. CMA has been purified from both normal and neoplastic colon carcinomas. Attempts to purify CMA from colon carcinomas grown as xenogeneic grafts in hamsters and nude mice have given poor results. A new purification procedure is being developed for these tissue sources. The procedure involves phenol-water partition extraction, fractional ethanol precipitation, enzymatic digestion with pronase and DNase, and finally hydroxyapatite adsorption chromatography. An antiserum prepared against normal tissue derived CMA, and appropriately absorbed with mucinous ovarian cystadenocarcinoma fluid, had reactivity limited to the colon, when tested by immunoperoxidase against various normal adult tissue specimens. In an analysis of CMA in neoplasia, 23 out of 27 colon carcinomas were stained by the specific antiserum. Staining was found to correlate with tumor differentiation: 22 out of 23 well and moderately differentiated tumors were positive. Extracolonic neoplasms are currently being examined for the presence of the antigen. A radioimmunoassay for the detection of CMA in body fluids is being developed.

Significance to Cancer Research: Purification of tumor-associated antigens for use in diagnostic testing and evaluation of immunologic assays, particularly radioimmunoassays, is highly relevant to cancer immunodiagnosis.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $103,386

CONTRACT RESEARCH SUMMARY

Title: Immunohistochemical Studies of Tumor-associated Antigens

Principal Investigator: Dr. F. James Primus
Performing Organization: University of Kentucky
City and State: Lexington, KY

Contract Number: NO1-CB-84257
Starting Date: 9/18/78 Expiration Date: 9/17/81

Goal: To isolate and characterize cancer-related markers in or on tumor cells by immunohistochemical techniques and describe their relationship to the discrimination of neoplastic cells from normal cells.

Approach: Studies will be performed using colon carcinoma antigen III (CCA-III), CSAp, β-HCG, Regan isoenzyme, and major blood group antigens in an attempt to discriminate between malignant and nonmalignant cells and to determine whether immunocytochemical methods can accurately detect metastatic spread in regional nodes.

Progress: Using an indirect antibody-conjugated immunoperoxidase procedure, β-HCG has not been detected in formalin-paraffin specimens from breast (42 cases), gastrointestinal, including stomach and colon (35 cases), and ovarian cancer (5 cases) having diverse histopathology. Staining of 25 cases of primary colonic adenocarcinomas revealed the same incidence of positivity (88%) and similar cytological distribution for both CEA and CCA-III. Uninvolved, morphologically normal mucosa stained similarly (50%) for both antigens, indicating the same degree of colon tumor cell specificity for each antigen. CEA staining was as accurate as histopathology in detecting micrometastatic spread. The indirect, conjugated antibody test was found superior to the triple-bridge procedure in staining for CEA, but was similar in sensitivity to the PAP method on specimens containing greater than 17 μg CEA/g. Staining of uninvolved colonic mucosa from malignant and nonmalignant disease specimens was greater with PAP (94%) than with the indirect procedure (53%). Method of colon tissue fixation, formalin or ethanol, prior to paraffin embedding revealed two different staining patterns for CEA. Staining for CSAp was only obtained in frozen or ethanol fixed, paraffin-embedded specimens, necessitating a prospective study of this antigen. CSAp is a normal constituent of colonic goblet cells and, in the few cases studied, colonic adenomas are highly positive whereas adenocarcinomas have reduced staining which appears as scattered intraepithelial lakes within the glandular lumen.

Significance to Cancer Research: The immunohistochemical studies of well-known or well-defined tumor-associated antigens in tissue sections may be useful in the diagnosis or prognostic evaluation of cancer.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $74,961

CONTRACT RESEARCH SUMMARY

Title: Development of Immunoglobulins Labeled with Gamma-emitting Radioisotopes

Principal Investigator: Dr. David M. Goldenberg
Performing Organization: University of Kentucky Research Foundation
City and State: Lexington, KY

Contract Number: NO1-CB-64011
Starting Date: 6/30/76 Expiration Date: 9/30/80

Goal: To detect and localize, within the region of the body, cancer tissue too small to be found with currently available methods.

Approach: Detection and localization of early and/or small cancer lesions will be improved by development of immunoglobulins which are relatively specific to selected human tumor tissue components and which are labeled with suitable gamma-emitters to provide external localization of the tumor site. During the first year the tumor localizing efficiency of ^{131}I-labeled anti-CEA immunoglobulins will be evaluated in animal studies and then tested in human subjects. Clinical testing may begin before the end of the first year and will be done first with advanced incurable cancer patients, then with those with curable cancer, and with those having questionable cancer. Other radiolocalization systems based upon non-CEA tumor antigens will also be studied. However, since these systems are in earlier stages of development, no clinical trials will be made on them before the third year of the study.

Progress: ^{131}I-labeled anti-CEA immunoglobulin has been purified and tested for purity and suitability for human use. Clinical testing began in the summer of 1977, and by May 1979 approximately 150 cancer patients were studied. After computer subtraction of blood pool background activity, localization of the labeled immunoglobulin could be detected, at 48 hr post-injection, in most of the cases studied. CEA-containing tumors at various body sites could be localized by external scintillation techniques. Scanning results were negative in patients without demonstrable tumors or with tumors apparently free of CEA. Careful analysis of CEA radioimmunodetection in patients with colorectal or ovarian carcinoma has revealed that the method has an accuracy of over 90%, based upon tumor sites (primary and secondary) confirmed by other methods. Certain tumor sites could be demonstrated by radioimmunodetection and later confirmed by other techniques (including surgery) although these sites were not detected by other standard diagnostic measures, including colonoscopy/GI series, ultrasonography, and abdominal computed tomography. Very high plasma CEA titers in some of the patients did not appear to prevent successful radioimmunodetection, even when evidence was obtained supporting the formation of CEA-antibody complexes in the blood.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Screening Technique for Blood in Stool To Detect Early Cancer of Bowel

Principal Investigator: Dr. Victor A. Gilbertsen
Performing Organization: University of Minnesota
City and State: Minneapolis, MN

Contract Number: NO1-CB-53862
Starting Date: 6/30/75 Expiration Date: 6/29/81

Goal: To demonstrate a significant reduction in mortality due to colorectal cancer between the annually screened and the control group by employing the Hemoccult (R) form of the guaiac test for stool blood in combination with a diagnostic protocol for the source of bleeding.

Approach: Forty-five thousand participants between the ages of 50 and 80 years residing in the state of Minnesota are randomly allocated to three groups on the basis of age, sex and geographic location in the state. Slides are completed after the observance of a meat-free diet with suggested high fiber content. One of the groups completes the slides once per year, another every other year, and a third group which is unscreened serves as the control. Each slide set consists of 6 slides representing two from each of three consecutive stools. Participants submitting samples positive for blood receive a diagnostic examination (including a colonoscopy, UGI series, and proctoscopy) to determine the cause of bleeding. All data including pathology reports on biopsy material and surgical specimens are computerized for easy retrieval. Extensive follow-up procedures are designed to retain participation of all subjects throughout the five years of testing and an additional five years of follow-up. Extensive dietary and health history data are collected from each subject via questionnaire.

Progress: Significant progress to date includes completion of the first screen (both groups screened) and determination that 8% of the first screenees submitting at least 1 slide positive for occult blood and examined at the University of Minnesota (84%) were found to have at least one GI malignancy. The 16% examined elsewhere under a variety of protocols (most not including colonoscopy) have been accounted for and found to have a rate of 9% GI malignancies. The comparability of the two rates is being studied while the second and third screens are being completed. The diagnostic protocol for detection of the source of bleeding has been modified to exclude the single column B.E. x-ray subsequent to data indicating the superior uniformity of results using colonoscopic examination with air contrast B.E. x-ray (in those cases in which the cecum is not reached). Extensive follow-up procedures are followed to maximize continued participation and minimize attrition over the five-year screening and five-year follow-up periods.

Project Officer: Mr. Louis P. Greenberg
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $676,257

CONTRACT RESEARCH SUMMARY

Title: Antigens on Human Lymphoid Organs: Immunodiagnosis of Leukemias and Lymphomas

Principal Investigator: Dr. John Kersey
Performing Organization: University of Minnesota
City and State: Minneapolis, MN

Contract Number: NO1-CB-84261
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: To evaluate lymphoid differentiation antigens, particularly those on bone marrow and thymus cells, as possible immunodiagnostic markers for leukemia and lymphomas, and as markers for functional lymphoid cell combinations.

Approach: Produce monoclonal antibodies from hybridomas against T-lymphocyte associated antigens and the ALL antigen. Use these antibodies to evaluate malignant lymphoid cells for presence of these antigens. Particular emphasis will be placed on thymus antigens TA1, TA2 and TA3.

Progress: The following studies were continued during the past year: Cell Antibodies. (1) Developed improved xenoantisera for characterization of human T lymphocytes; (2) produced human T cell antibodies by immunization of horses with thymocytes; (3) produced anti-T antisera in large quantities. Extensive cytotoxicity assays were performed, demonstrating cytotoxicity for T lymphocytes up to a dilution of 1:640. The horse anti-human T antibody was absorbed using placenta obtained at cesarean section. The placenta absorbed antibody was further tested and found to be cytotoxic for T lymphocytes at 1:320 but of interest showed no inhibition of colony forming units-committed (CFUc) up to a concentration of 1:40. These results demonstrate an antibody that spares cells committed to myeloid and monocyte differentiation but will react with cells committed to T lymphoid differentiation.

Differentiation Probes Using Lectin. Use of plant lectins as probes of lymphocyte differentiation has been started. Results indicate reactivity of FITC-PNA and FITC-SBA with 50-100% of thymocytes of fetuses and children in humans, 100% of thymocytes in mice, 4-23% of tonsillar lymphocytes, 0% of peripheral blood lymphocytes, 100% of monocytes-macrophages, 60-100% adherent bone marrow cells, and 0% of PHA stimulated lymphocytes. Of 23 non-T, non-B acute leukemias evaluated, 6 were found to have lectin receptors.

Pre-B Lymphocytes. Studies have continued on cells early in the differentiation along the B-axis. Several cases of chronic myelogenous leukemia have been evaluated in which the patients developed blastic crisis in the terminal phase of the disease. It was also noted that in these Phl^+ lymphoblastic leukemias all three had the ALL antigen, and two of the three (including the NALM-1 cell line) were cytoplasmic Ig^+.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $48,100

CONTRACT RESEARCH SUMMARY

Title: Detection of Tumor-specific Antigens in the Circulation

Principal Investigator: Dr. R. W. Baldwin
Performing Organization: University of Nottingham
City and State: Nottingham, England

Contract Number: NO1-CB-74167
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Investigate the diagnostic applicability of circulating tumor-associated antigens.

Approach: Follow the levels of circulating antigens during tumor growth in a variety of situations, attempting to relate measured levels of antigen to tumor size. A wide range of rat tumors (3-methylcholanthrene-induced sarcomas, hepatocellular carcinomas, and spontaneously arising mammary adenocarcinomas in Wistar rats) will be used.

Progress: Completed studies show that serum-borne tumor antigen can be detected using a membrane immunofluorescence inhibition assay well before subcutaneous or intramuscular growth of a transplanted rat hepatoma, D23, becomes palpable. The next phase of the studies now being developed is designed to evaluate whether circulating tumor antigen can be used to detect early growth of a spontaneous mammary carcinoma Sp4 following implantation of low inocula of tumor cells into the mammary pad, and to detect the development of both regional lymph node and pulmonary metastases following surgical resection of the mammary pad growths of tumor Sp4. Attention has been directed toward the development of cell-free radioimmunoassays and cell binding tests. A critical requirement for this approach is a good source of tumor-specific antibody. This has been provided by our related studies which have led to the development of a monoclonal antibody by hybrids between P3-NS1 mouse myeloma cells and spleen cells from Sp4-immune rats. The monoclonal Sp4 antibody has now been purified by affinity chromatography on Sp4 immobilized cells and this preparation reacts positively in a ^{125}I-antiglobulin binding assay with Sp4 target cells at a dilution of 1/5000. The binding capacity of monoclonal anti-Sp4 to solubilized and immunoabsorbent purified Sp4 antigen has also been evaluated and a co-precipitation assay for tumor antigen assay is being finalized. These assays are next to be used to determine circulating Sp4 antigen in sera from rats bearing either primary or metastatic deposits of mammary carcinoma Sp4.

Significance to Cancer Research: These studies will establish in animal systems the value of monitoring circulating tumor antigen for diagnosis.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Development of Serum Isozyme Test for Human Cancer Patients

Principal Investigator: Dr. K. C. Tsou
Performing Organization: University of Pennsylvania
City and State: Philadelphia, PA

Contract Number: NO1-CB-64029
Starting Date: 6/30/76 Expiration Date: 6/30/80

Goal: To improve on the early diagnosis of cancer of the liver and further examine the possibilities of preventing the spread of primary cancers to other body sites with concentration on liver tissue.

Approach: (1) Further technical improvement of the 5'-nucleotide phosphodiesterase isozyme method as a diagnostic tool; (2) development of correlation data (biopsy, scan) and 5'-nucleotide phosphodiesterase-V for primary hepatoma and liver metastasis; (3) investigation of relationships between the isozyme and other known liver markers related to hepatic cancer; (4) improve the sensitivity of the test through other alternate assay methods; (5) correlation of clinical information and other liver function tests; and (6) suggest how this test can be best used for early diagnosis.

Progress: Progress in using 5'-nucleotide phosphodiesterase isozyme V (5'-NPD-V) for the detection of liver metastasis has been made in methodology development and sampling study. An alternate method to detect isozymes based on 4-methylumbelliferone 5'-thymidine phosphodiester as a substrate can be done in about 3 hours from the time of blood drawing. With the total samples of 8,000, the incidence for positive 5'-NPD-V among patients with confirmed liver metastases has remained at 85-95% range for all forms of cancer. Thus, this is a valuable test for confirming liver metastases. In 4 cases of breast cancer, a study between alkaline phosphatase and 5'-NPD-V revealed that if alkaline phosphatase is consistently elevated and 5'-NPD-V is negative in 6 months follow-up, these patients only had bone metastases.

Prospective study on 400 melanoma patients has been initiated. Liver scan data were available in 167 patients. Nine out of ten patients with positive scans were positive for 5'-NPD-V. The patient with positive scan and negative 5'-NPD-V had negative liver biopsy. Forty-two patients had positive 5'-NPD-V and negative scan. The remaining 115 patients were both negative in 5'-NPD-V and liver scan ($\chi^2=14.86$, $p<.001$). Follow-up scan on patients with positive 5'-NPD-V indicated liver metastasis in one patient that had a lead time of at least 9 months for 5'-NPD-V. All other liver function tests (LFT's) were normal for this patient. Follow-up 5'-NPD-V and liver scan on the 157 patients with initial negative scan are still in progress. Thus far, 5'-NPD-V can be a valuable diagnostic aid in predicting liver metastasis if used in follow-up patients on a protracted period.

Project Officer: Mr. Louis P. Greenberg
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Slit-Scan Technique as Cancer Prescreening Automated System in Cytology

Principal Investigator:	Dr. Leon L. Wheeless, Jr.
Performing Organization:	University of Rochester
City and State:	Rochester, NY

Contract Number: N01-CB-33862
Starting Date: 3/19/73 Expiration Date: 3/18/82

Goal: Automation of clinical cytologic screening and diagnosis.

Approach: The contractor will evaluate the performance characteristics of the X-Y-Z Slit-Scan Flow System. A benchmark study of the X-Y-Z system will be performed to (1) determine system characteristics including rate and causes of remaining false alarms and (2) document true alarm rates for abnormal specimens. Correlation studies will continue and studies on second stage processing techniques will begin.

Progress: The X-Y-Z Slit-Scan Flow System has been fabricated and testing initiated. Evaluation of system performance characteristics has begun. Cell contours have been recorded in X, Y, and Z dimensions in flow validating the concept and basic design. The static cell data base has been expanded and a flow benchmark study will begin shortly. The true alarm rate for abnormal specimens has been documented on the flow correlating system. Sample preparation protocols continue to be evaluated and improved.

Project Officer: Dr. Bill Bunnag
Program: Diagnosis Branch
Technical Review Group: Cytology Automation Committee
FY 80 Funds: $403,939

CONTRACT RESEARCH SUMMARY

Title: Detection and Characterization of Soluble Antigen-antibody Complexes in the Circulation

Principal Investigator: Dr. Harold Wanebo
Performing Organization: University of Virginia
City and State: Charlottesville, VA

Contract Number: NO1-CB-84262
Starting Date: 9/28/78 Expiration Date: 9/27/81

Goal: Determine the diagnostic significance of circulating antigen-antibody complexes in the sera of cancer patients, identify the source of the antigen, and develop a sensitive assay for its presence.

Approach: Compare the results of the Raji cell radioimmunoassay, Clq binding radioimmunoassay, and mixed cryoglobulins for measurement of serum immune complexes, and determine the utility of measuring these complexes in monitoring cancer patients under various therapies. Isolate and characterize these complexes.

Progress: Patients with carcinoma of colon and breast and melanoma were studied. The Raji RIA was the most sensitive IC assay. In 65% of 17 patients with primary colonic cancer, and in 64% of 14 with metastatic disease, circulating IC were detected by at least one method. The Raji RIA was positive in 58% compared with 19% for the Dlq BA and 13% for cryoglobulins. Coincident with presumed curative surgery in the primary group was a fall in the level of circulating IC. When carcinoembryonic antigen levels exceeded 20 ng/ml (normal 2.5 ng/ml), Raji RIA positivity was 9%. Similarly, IC were detected by at least one method in 44% of 18 patients with malignant breast lesions. In the early postoperative period (mean 2.2 months) following presumed curative surgery, an increase in incidence of circulating IC in 68% was followed by a fall to 36% in the late postoperative period (mean 7.5 months). The mean Raji RIA values for the time periods were 17, 37 and 18 μg equiv. AHG/ml. When patients with benign breast disease (predominantly fibrocystic disease) were studied, there was a similar 44% incidence of IC with a mean Raji RIA value of 16 μg equiv. AHG/ml. Eighteen patients with surgically treated melanoma are being followed. In the initial sample, IC were detected in 28%, all with a positive Raji RIA and one with an elevated cryoglobulin. All IC values either fell or normalized with treatment. In the 12 months of follow-up 56% of patients have developed circulating IC without clinical disease recurrence. Nine rabbits (3 colon, 5 breast, 1 melanoma) were immunized with Raji cells containing bound IC. Following adsorption of the rabbit antisera against Raji cells and human AB erythrocytes, two of four with breast cancer antisera tested to date reacted with both autologous and homologous tumors. Studies are under way to determine if the rabbit antisera react with known tumor antigens.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: $108,227

CONTRACT RESEARCH SUMMARY

Title: Study of a Serum Alkaline Phosphatase Variant (FHAP) in Cancer Patients

Principal Investigator: Dr. Frank C. Larson
Performing Organization: University of Wisconsin
City and State: Madison, WI

Contract Number: N01-CB-74173
Starting Date: 9/15/77 Expiration Date: 9/14/80

Goal: To define the diagnostic and prognostic significance of an isoenzyme of alkaline phosphatase (FHAP) in serum of human cancer patients.

Approach: A column separation technique was perfected which permits quantitative assay of FHAP. Patients with various types of cancers are studied to determine the relationship between serum FHAP and the presence of, the amount of, and the type of cancer. These data are compared with normal persons and patients with various benign diseases. Studies are underway in which the diagnostic usefulness of FHAP is compared with other cancer markers and which relate changes in serum FHAP to the course of cancer under treatment.

Progress: Studies have been completed which demonstrate a positive relationship between the presence of cancer and the size of the tumor load and the FHAP activity in serum. In one study three groups were compared.

	FHAP in U/L		
Group	mean	median	% > 2
Normal (N=931)	1.02	0.91	4.0
Benign disease (N=466)	1.52	1.07	11.0
Cancer (N=343)	9.20	2.31	60.0

An analysis of a panel of sera provided by the Mayo Clinic revealed:

Group	No. 2.0 U/L FHAP	%
Normal (N=30)	0	0
Smokers (N=30)	1	1
Benign (N=60)	12	10
Cancer pancreas (N=30)	24	80
Cancer gyn (N=29)	14	50
Cancer lung (N=30)	18	60

Pancreatic pseudocyst accounted for a large number of false-positive results in the Mayo Group. Ovarian carcinoma accounted for most of the false-negative gyn cancer results. A study comparing the sensitivity of and specificity of FHAP and CEA in various tumors is near completion. A study of the relationship of FHAP values to the course of cancer is continuing.

Project Officer: Mr. Louis P. Greenberg
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: New Methods for Isolation and Characterization of Human PBLs

Principal Investigator: Dr. Hans Wigzell
Performing Organization: Uppsala University
City and State: Uppsala, Sweden

Contract Number: NO1-CB-74129
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To develop new methods to isolate and characterize human peripheral blood lymphocytes (PBLs).

Approach: Obtain unique markers, morphological or functional, specific for a given subgroup of mononuclear cells by use of classical and innovative cell purification techniques and other methods.

Progress: Monoclonal hybridomas making antibodies against human mononuclear cells have been prepared or are in preparation. Some of these hybridomas seem to make antibodies unique for T cell subpopulations and also for activated T cells in contrast to resting T lymphocytes. Lines of NK cells have been prepared in vitro to allow a successful analysis of markers on activated NK cells versus normal NK cells. Data obtained indicate differences between the two subsets of cells. Human resting T cells can readily start to express HLA-DR antigens upon activation but the antigens so far analyzed have all behaved like the DR structures found on B lymphocytes, that is, no human I-J analogue has been found so far. A search for subpopulations of T cells with regard to Fc receptors for various Ig classes has revealed the existence of a low percentage of T cells with simultaneous display of receptors for IgM and IgG. It is not known whether observed T cells with receptors for IgD and IgA have similar "double" receptors. Helper T cell clones have been established in parallel with killer T cells from the same donor to allow a comparison of surface glycoprotein patterns of the functionally distinct subpopulations of cells. A similar comparison is also being made between IgM and IgG-binding T cells. In testing lectins for their ability to define subpopulations of functional human mononuclear cells, the most discrete but also most variable results have been obtained with the N-acetylgalactose amine reactive Vicia villosa lectin, showing that in some but not all MLCs the CTL (cytolytic T cells) express receptors for the lectin in a select manner. The distribution pattern of complement receptors on human lymphocytes has been further pursued: some 11% bind C3b, 6% C3bi, and around 2% C3d. The cells with receptors for C3b did normally fail to express receptors for the other two C3 breakdown products. Cells with ADCC ability could be found within the C3 breakdown binding lymphocytes. Presence of C3bi on the target cells constituted the most potent enhancing complement factor with regard to IgG induced ADCC.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Purification of Breast Tumor-associated Antigens

Principal Investigator: Dr. Robert K. Oldham
Performing Organization: Vanderbilt University
City and State: Nashville, TN

Contract Number: NO1-CB-74162
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Develop an assay for new tumor-associated antigens.

Approach: Isolate and purify the TAA of MCF-7, a breast cancer-derived tissue culture line, to generate heteroantisera for development of a radioimmunoassay for that antigen. Antigen isolation and purification are to be first analyzed with respect to antigen specificity in the leukocyte migration inhibition assay.

Progress: Plasma membrane preparations, tissue culture fluids, and 3M KCl extracts of MCF-7 have been shown to be immunologically active in the direct agarose micro-droplet migration inhibition assay using leukocytes from breast cancer patients as compared to normal controls. Of the first 100 patients with breast cancer tested with 3M KCl extracts, 70 were positive (migration index <0.9). None of the normals showed migration inhibition less than 0.9 with the same extracts. When normal women with a family history were tested, no response was seen ($MI < 0.9$) while almost 60% of women with biopsy proven premalignant breast disease showed inhibition ($MI < 0.9$). When tissue culture fluids were used as a source of antigen, similar data were observed. None of the normal individuals had migration inhibited to less than 0.95 while 35 of 48 (73%) of patients with breast cancer gave a positive test ($MI<0.9$). As before, none of the women with a family history of breast cancer had a positive test, of which 9 of 16 (56%) with biopsy proven premalignant condition were positive ($MI<0.9$). Preliminary fractionation has indicated that 1) the antigen is a glycoprotein; 2) the molecular weight is approximately 70,000 daltons; it can be recovered from an isoelectric focusing column in the pH 4.2 to 5.6 range. A preliminary heteroantiserum has been prepared in rabbits and has been shown to bind to a surface antigen on MCF-7 as determined by ^{125}I staph. protein A assay.

Significance to Cancer Research: Isolation of TAA and the development of a radioimmunoassay for carcinoma of the breast would be a valuable diagnostic and prognostic tool.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cell-mediated Reactivity of Normal Individuals to Human Tumor-associated Antigens

Principal Investigator: Dr. Robert K. Oldham
Performing Organization: Vanderbilt University
City and State: Nashville, TN

Contract Number: NO1-CB-74166
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Investigate effector cell subpopulations involved in natural cytotoxicity, the antigens to which this activity is directed, and the relationship of natural activity to the immune response directed at tumor-associated antigens.

Approach: Define the natural cytotoxic activity of the K-562 model system; characterize the effector cell; investigate the model system antigens.

Progress: A kinetic method of analysis of specificity in human spontaneous cell-mediated cytotoxicity has been developed. Data from inhibition experiments in which unlabeled inhibitory cells are cocultured with ^{51}Cr-labeled target cells and Ficoll-Hypaque separated mononuclear human peripheral blood leukocytes are analyzed in a manner analogous to the inhibition of enzyme-substrate reactions. Using the Lineweaver-Burke transformation of the Michaelis-Menten equation, a reciprocal plot of the velocity (number of cells lysed per unit of time) as a function of the initial substrate concentration (number of labeled targets cells) can be developed for each inhibitory cell line. If these plots, at different concentrations of inhibitory cells, have the same value for the maximal velocity, then the inhibition is defined as competitive and is interpreted to be the result of competition between determinants on the inhibitor cells and the target cells for the same receptor sites on the effector cells. A dissociation constant for each effector cell-target cell-inhibitor cell combination can be determined using Dixon plots of the inhibitor cell concentrations versus the reciprocal of the velocity at several substrate concentrations. The value of this constant allows a quantitative measurement of the affinity of receptor and ligand. These methods were used to test a panel of target cells against effector cell donors to determine specificity of natural cytotoxicity in terms of target cells and effector cells. Experiments have demonstrated that natural cytotoxicity in man consists of at least two specificities as evidenced by the mutual lack of competitive inhibition between F-265 and K-562 cell lines.

Significance to Cancer Research: An understanding of the mechanism and target antigen system of natural cytotoxicity is necessary to understand the biologic significance of this phenomenon and to quantitate its contribution and relationship to in vitro assays of tumor immunity.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Evaluation of the LAI Assay for Malignant Disease

Principal Investigator: Dr. Arthur Vandenbark
Performing Organization: Veterans Administration Medical Center
City and State: Portland, OR

Contract Number: YO1-CB-80316
Starting Date: 9/30/78 Expiration Date: 9/29/80

Goal: To objectively evaluate the utility of the leukocyte adherence inhibition (LAI) assay in the monitoring of progress of cancer patients under treatment and further improve the assay for detection of reactivity.

Approach: In a double blind study, test the specificity and frequency of positive LAI reactivity to various tumor extracts in patients with breast, lung, colon, and squamous cell carcinoma, using individuals with benign diseases and normal persons as controls. Perform longitudinal studies in LAI response during course of cancer therapy.

Progress: The contractor has prepared 3M KCl extracts from colon, lung, squamous, and breast tumors and has tested these extracts over a wide range of concentrations in 150 cancer patients, 20 benign disease controls, and 107 normal individuals. He has enrolled and evaluated in blind fashion 37 breast patients, 17 colon patients, 28 squamous patients, and 22 lung patients. In general, most tumor extracts can discriminate tumor patient responses versus normal control responses, but different tumor extracts of the same histologic type vary in their ability to discriminate responses of patients in different tumor groups. Thus far, at least one breast carcinoma and one colon carcinoma extract which elicits a significantly higher percent positive reactivity in the homologous tumor patient group than in patients with other tumors have been found. A battery of extracts is being prepared from five breast carcinomas, two benign breast tumors, one metastatic breast tumor and two normal breast tissues in order to improve the evaluation of patient responses versus other cancer, benign and normal control responses. If this approach increases the ability to discriminate between breast cancer and other cancer patients, a similar approach will be utilized with the other tumor types.

Significance to Cancer Research: The objective analyses of the leukocyte adherence inhibition assay will determine its possible utility as an immunodiagnostic test for cancer.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human PBLs

Principal Investigator: Dr. Nathan Sharon
Performing Organization: Weizmann Institute
City and State: Rehovot, Israel

Contract Number: NO1-CB-74163
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Develop new methods for isolating and characterizing human peripheral blood lymphocytes (PBLs).

Approach: Develop procedures for cell fractionation by selective agglutination with lectins. Mononuclear cells from both healthy donors and cancer patients will be screened for their ability to bind fluorescent derivatives of peanut, soybean and wheat germ agglutinins.

Progress: This contract is completing its second year. Recent work has focused on applying the lectin-binding and cell-fractionation techniques, worked out in the murine system, to human thymocytes. The majority (60-80%) of the latter cells bound peanut agglutinin (PNA); upon removal of sialic acid by sialidase, all cells bound the lectin. PNA^+ cells, isolated by selective agglutination with the lectin, responded poorly to mitogenic stimulation by PHA and in the mixed lymphocyte reaction whereas the PNA^- cell responded well to both stimuli. It seems, therefore, that human PNA^+ thymocytes, like the corresponding murine cells, are functionally immature and that the lectin provides a unique tool for separating mature from immature thymocytes. PNA^+ thymocytes bear the receptor for E-rosette formation, suggesting that the masking of the lectin receptor by sialic acid occurs at a later stage of T-cell maturation than the expression of the E-rosette receptor. PNA is thus a more sensitive marker of T-cell immaturity than E-rosetting. A PNA^+ fraction, which responds poorly to PHA and Con A as compared to the PNA^- fraction, was isolated from human cord blood. In studies on human peripheral blood mononuclear cells (PBL), less than 2% of the lymphocytes from normal donors bound PNA. In leukemic patients, the type of leukemia determined the extent of binding: in ALL, stem cell leukemia, and myeloid leukemia, most of the cells of greater than 50% of the patients were PNA^+, while cells from more than 90% of CLL patients were PNA^-. Studies are in progress on the use of soybean agglutinin (SBA) for the separation of T and B cells from human peripheral blood. These studies include characterization of the separated cell populations by surface markers and immunobiological tests.

Significance to Cancer Research: The presence of PNA receptors or the blastic cells of patients with acute leukemia could be of therapeutic importance, since they may serve as selective targets for PNA-bound drugs.

Project Officer: Ms. Judith M. Whalen
Program: Diagnosis Branch
Technical Review Group: Tumor Immunology Committee
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Development of Large Area Solid State Image Receptors for X-Ray Imaging

Principal Investigator: Dr. Moshe Ein-Gal
Performing Organization: Xerox Corporation
City and State: Pasadena, CA

Contract Number: N01-CB-74211
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To improve clinical diagnostic radiology and to provide for the diagnosis of early cancer by developing a computerized imaging system based on a large area solid state x-ray receptor superior in clinical usefulness to those currently available.

Approach: A large area solid state x-ray detector will be produced which will transform the x-ray flux into a digitally stored high quality image. The system to be developed, an x-ray selenium electronic linear scanner, will use the capability of a selenium alloy photoreceptor to create a latent image from absorbed x-rays. The latent image, a distribution of electric charge on the photoreceptor surface, will be detected by a microelectrometer scanning arrangement. Signals from the electrometer array will be coupled with the necessary control, display, and image storage memory components. Feasibility of the imaging system components will be developed in the first year. Further design, development, and optimization of the system will be completed during the second year. Clinical evaluation of the total imaging system will be conducted during the third year as well as further improvements and optimizations.

Progress: Initial assembly and feasibility testing of the detection components were accomplished during the first year as scheduled. The computer system, the dedicated image processing hardware and the x-ray facilities were installed during the second year of the contract. Since the beginning of the third year, the system has been prepared for hospital installation, a cassette scanner has been designed and a high resolution (1024^2) display unit has been fabricated. A team of radiologists for the clinical evaluation has been selected and the system was moved into the hospital during April 1980. Clinical testing currently is in progress.

Project Officer: Dr. R. Q. Blackwell
Program: Diagnosis Branch
Technical Review Group: Diagnostic Research Advisory Group
FY 80 Funds: $300,000

CONTRACT RESEARCH SUMMARY

Title: Maintain an Animal Holding Facility and Provide Attendant Research Services

Principal Investigator: Dr. Diane Hernandez
Performing Organization: Cor Bel Laboratories, Inc.
City and State: Rockville, MD

Contract Number: NO1-CB-64024
Starting Date: 11/1/75 Expiration Date: 10/31/79

Goal: Maintain colonies of inbred mice (7,500 animals), inbred rats (300 animals), and rabbits (25 animals) and carry out selected breeding protocols with these animals as specified by the project officer. These animals are to be maintained in support of intramural research programs in the Immunology Branch, NCI.

Approach: Colonies of mice, rats, and rabbits are to be housed and fed according to National Research Council standards. Technical manipulations and breeding are to be carried out as directed by the project officer.

Progress: Performance on this contract has been highly satisfactory. The animal colonies have been established and are being maintained according to National Research Council standards. Animal health has, in general, been excellent, and breeding protocols have been satisfactory. Record keeping and transferring of animals to and from the NIH campus have all been satisfactory.

Significance to Cancer Research: This animal colony is necessary in support of intramural research programs in the Immunology Branch of NCI. Many of these programs are concerned with the immune response to cancer.

Project Officer: Dr. David H. Sachs
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad hoc Review)
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Administrative Support Services

Principal Investigator: Ms. Karen Meinzen
Performing Organization: CSR, Inc.
City and State: Washington, D.C.

Contract Number: 263-79-C-0021
Starting Date: 10/19/79 Expiration Date: 10/18/80

Goal: To support administrative staff in preparation of scientific meetings and the research components in accomplishing their administrative tasks.

Approach: Current staffing situation places impossible demands on the administrative staff and committee chairmen (presently non-NCI scientists) in carrying out their responsibilities. This project is to support such routine tasks.

Progress: The contractor supported efforts in organizing 10 conferences and workshops. The support included preparation of pre- and post conference technical material requiring editorical and printing activities.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: DCBD
Technical Review Group: Ad Hoc Committee
FY 80 Funds: $400,000

CONTRACT RESEARCH SUMMARY

Title: NCI Histocompatibility Typing Center

Principal Investigator: Dr. A. H. Johnson
Performing Organization: Duke University
City and State: Durham, NC

Contract Number: NO1-CB-53890
Starting Date: 4/1/79 Expiration Date: 3/31/80

Goal: To study HL-A, A, B, C, DR, cell-surface antigens on patients and in normal individuals to attempt to establish a correlation between a particular antigen and a disease condition. Families in which more than one member has a particular disease will be studied to determine if the major histocompatibility complex contributes to the disease state.

Approach: Complement dependent cytotoxicity studies will be performed with cell and serum provided by the project officer.

Progress: HLA, A, B, C, DR typings were performed on a panel of normal individuals and individuals with the following disease diagnoses: diabetes mellitus with and without insulin allergy, systemic lupus erythematosus, Sicca syndrome, acute lymphocytic leukemia, Hodgkin's disease, and malignant melanoma. The significant findings are as follows: Patients with the Sicca syndrome demonstrated an increased frequency of the DRW-3 antigen compared to the normal population. In comparing this disease population to patients with Sicca syndrome with rheumatoid arthritis, the DRW 3 antigen was replaced by DRW 4. Thus, it appears that the genes of the major histocompatibility complex influence the manifestation of the disease syndrome. Patients with systemic lupus erythematosus were found to have elevated DRW-2, DRW-3 antigens compared to the normal population and, in addition, one antisera IA 715 reacted with 75% of the patients compared to 14% of the control population. This disease appears to be of autoimmune etiology. The antigen detected by the antisera IA 715 may mark immune response genes. Analysis of data generated in typing families in which disease association has been determined has resulted in important conclusions related to the genetics of the antigens in the DR region of the major histocompatibility complex. Studies performed thus far indicate that several gene loci control the expression of these antigens, a finding which contributes to the knowlege of the genetics of the MHC.

Significance to Cancer Research: Establishing correlations of antigens controlled by genes of the major histocompatibility complex has suggested a fundamental biological role for these genes in disease manifestations. This information establishes the concept of immune response genes in man. As cancer is a complex biologic entity, the investigation and identification of immune response genes potentially adds important information in our understanding of the lack of immune response to neoplastic transformation.

Project Officer: Dean L. Mann, M.D.
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Maintenance and Development of Inbred and Congenic Resistant Mouse Strains

Principal Investigator: Martha J. McGowan
Performing Organization: Litton Bionetics, Inc.
City and State: Kensington, MD

Contract Number: NO1-CB-94325
Starting Date: 2/1/79 Expiration Date: 1/31/82

Goal: To maintain a colony of inbred pedigreed strains of mice which are needed to support ongoing NCI intramural research in transplantation immunology.

Approach: The contractor maintains a colony of approximately 40 special inbred and congenic resistant strains of mice by pedigreed brother-sister mating. Quality control testing is carried out at each generation by cytotoxicity typing of animals from each strain. Alloantisera are raised between mouse strains to assist in this quality control typing and sera and animals are shipped by the contractor to collaborating investigators at NIH and elsewhere.

Progress: The contractor has maintained all inbred and congenic resistant strains of mice in excellent condition. Breeding of each strain and of hybrid strains, record keeping, and quality control testing have all been highly satisfactory. A backcrossing program has been instituted for all congenic resistant strains in order to keep the backgrounds of these strains identical. This involves backcrossing of each congenic to the reference background line once every 6-10 generations. This program has also been very satisfactory to date. Three new recombinant H-2 haplotypes have been identified during the process of this backcrossing, and these have been bred to homozygosity and established as three new valuable inbred congenic strains.

Antisera for histocompatibility antigen typing have been prepared in a variety of combinations and have been found to be excellent reagents. A series of new strain-restricted typing sera have been produced in order to identify each strain in the colony and distinguish it from all other strains. Shipping of animals and sera to collaborating investigators at NIH and elsewhere has been very satisfactory. The animals shipped from these pedigreed colonies have generally been of excellent health and have provided breeding stock for the production of larger numbers of experimental animals in numerous laboratories.

Significance To Cancer Research: This animal facility is needed for the breeding and maintenance of these inbred congenic resistant strains of mice. These animals make possible research on individual histocompatibility antigens and, in particular, the role of the major histocompatibility complex in the transplantation of tissues and cells and in the immune response to cancer.

Project Officer: Dr. David H. Sachs
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 80 Funds: $239,953

CONTRACT RESEARCH SUMMARY

Title: Induction, Transplantation, and Preservation of Plasma Cell Tumors in Mice and the Maintenance of Special Strains

Principal Investigator: Martha J. McGowan
Performing Organization: Litton Bionetics, Inc.
City and State: Bethesda, MD

Contract Number: NO1-CB-94326
Starting Date: 3/1/79 Expiration Date: 1/31/82

Goal: Transplantation, preservation induction, and shipping of plasmacytomas, T- and B-cell lymphomas in mice. Breeding of rare strains of mice, hybrids, and wild mice.

Approach: To maintain a closed conventional colony of BALB/c, CB-20, CAL-20 mice, and other BALB/c sublines for use in plasmacytoma induction and for the maintenance of a bank of transplantable plasmacytomas and other lymphocytic tumors. The mice in this environment are suitable for long-term plasmacytoma induction studies and for the generation of congenic strains of the BALB/c background. New BALB/c congenic lines, carrying genes from the plasmacytomagenesis-resistant DBA/2 background, are being bred for use in plasmacytoma induction studies to identify genes involved with the genetic susceptibility of plasmacytomagenesis. In addition, to maintain a screening bank of myeloma proteins for the detection of new antigen binding myeloma and to maintain a wild mouse colony.

Progress: The contractor has maintained 2,000 tumor lines and has made 385 shipments to intramural and outside investigators. Approximately 15 different Balb/c congenics carrying DBA/2 genetic markers have been developed. Several of the genetic marker lines have been made homozygous for the DBA/2 allele at N6 and are being bred for induction studies which will determine whether that particular marker is involved in resistance to plasmacytomagenesis.

The wild mouse colony is producing sufficient numbers of Mus caroli, cervicolor, pahari, spretus, cookii, and Mus musculus subspecies for continuing studies on the genetics of immunoglobulins. Continued studies of the biological activity of myeloma proteins have produced new antigen binding myeloma proteins from different allotype backgrounds.

Significance to Cancer Research: Provides essential support for the study of plasmacytomagenesis (carcinogenesis) with the specific goal of determining the genetic basis of susceptibility to tumor induction by mineral oil. Supplies esential biological material for investigators studying the biology of neoplastic plasma cells, tumor immunology, the genetics of immunoglobulins, and immunoglobulin synthesis.

Project Officer: Dr. Michael Potter
Program: Tumor Immunology Program
Technical Review Group: Immunology Suport Contract (Ad Hoc Review)
FY 80 Funds: $424,950

CONTRACT RESEARCH SUMMARY

Title: Measurement of Immunological Reactivity to Human Cancer

Principal Investigator: Dr. Martin Padarathsingh
Performing Organization: Litton Bionetics, Inc.
City and State: Kensington, MD

Contract Number: NO1-CB-63975
Starting Date: 9/1/78 Expiration Date: 4/30/80

Goal: To study the immune responses of cancer patients to tumor-associated antigens and to provide support for the Laboratory of Immunodiagnosis, NCI, for its detailed studies of cell-mediated immunity to human cancer.

Approach: Immunological tests to evaluate and monitor the immunocompetence of patients and their response to tumor-associated antigens include lymphocyte stimulation by mitogens and autologous tumor antigens; rosette assays for enumeration of T cells; leukocyte migration inhibition assays against recall antigens and tumor-associated antigens; and studies of direct cytotoxicity and antibody-dependent cell-mediated cytotoxicity against various human tumor cell lines.

Progress: Since a considerable number of patients with carcinoma of the lung or breast have been studied on this project over the past several years, it was important to determine whether the test results correlated with clinical course of the disease. In lung cancer, depressed lymphoproliferative responses in mixed leukocyte cultures performed at about one month after surgery in stage I patients correlated with a high rate of development and recurrent disease. In breast cancer, a significantly higher rate of recurrence was associated with low or negative lymphoproliferative responses to autologous tumor antigens, with mixed leukocyte culture responses in the normal range, and with positive reactivity in the leukocyte migration inhibition assay after surgery. These results indicate that some of the immunologic tests performed as a part of this project may be useful in substaging patients with lung and breast cancer. Another emphasis in the project has been on the detailed monitoring of patients receiving immunotherapy with BCG or C. parvum. It was found that after one inoculation with either agent, there was a rapid increase in cytostatic activity of monocytes and natural killer cells and a concomitant depression in lymphoproliferative responses to PHA or in mixed leukocyte cultures. These studies appear to be promising in providing new information on the immunologic effects of immunotherapeutic manipulation.

Significance to Cancer Research: These studies are providing important information regarding the usefulness of various assays of cell-mediated immunity for immunodiagnosis and for monitoring the clinical course of cancer patients.

Project Officer: Dr. Ronald B. Herberman
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 80 Funds 0

CONTRACT RESEARCH SUMMARY

Title: Melanoma Cell Vaccine in In Vitro Assays for Humoral and Cellular Cytotoxicity

Principal Investigator: Dr. Grace Cannon
Performing Organization: Litton Bionetics, Inc.
City and State: Kensington, MD

Contract Number: NO1-CB-53916
Starting Date: 5/9/75 Expiration Date: 5/10/81

Goal: To prepare cultured melanoma cells for immunotherapy vaccine and carry out in vitro cytotoxicity tests to measure immune responses of patients to the vaccine.

Approach: (1) Grow human melanoma cells free of contaminants, treat cells with neuraminidase, freeze under sterile conditions, and provide cells as needed for human immunotherapy.

Progress: During FY 80 six new batches of pooled vaccine have been prepared from human melanoma cell lines, UCLA SM-6, UCLA SM-12 and UCLA SM-14. In addition 300 serum samples were processed and frozen.

Significance to Cancer Research: The melanoma cell line vaccine is being used in a clinical immunotherapy trial.

Project Officer: Dr. John R. Wunderlich
Program: Tumor Immunology Program
Technical Review Group: Immunology Suport Contract (Ad hoc Review)
FY 80: Funds: $135,348

CONTRACT RESEARCH SUMMARY

Title: Studies of the Immune Response of Mice and Rats to Tumor Antigens

Principal Investigator: Dr. Martin Padarathsingh
Performing Organization: Litton Bionetics, Inc.
City and State: Kensington, MD

Contract Number: NO1-CB-74093
Starting Date: 2/28/77 Expiration Date: 2/27/81

Goal: Maintenance of colony of mice and rats, to support study of natural cell-mediated cytotoxicity of mice and rats and of their immune responses to tumor-associated antigens. Studies on in vivo resistance to tumors and correlation with in vitro cellular immune responses.

Approach: Hold and provide animals to NCI, and perform in vivo experiments to support studies of the natural cell-mediated cytotoxicity against tumors of mice and rats.

Progress: During the past year, this contract has provided support mainly for studies in mice of the genetic factors regulating the activity of natural killer (NK) cells and of the in vivo role of NK cells in resisting tumor growth. F_1 hybrids were produced by mating of inbred mice with high and low NK activity. F_1 hybrids from SJL and A/HeJ parents, both with low NK activity, had low NK activity. This suggested that the two strains shared genes contributing to low NK activity. This was confirmed and found to be at one locus. Much emphasis has also been placed on the role of NK cells in the rapid in vivo clearance of isotopically labeled tumor cells, during the first four hours after intravenous challenge. Clearance of tumor cells, particularly from the lungs, has correlated well with levels of NK activity. Implants of estradiol caused a persistent and profound deficit in ability to clear the labeled tumor cells, paralleled by a selective depression in vitro in NK activvity, but little or no effect on cytotoxic reactivity by T cells or macrophages. Urethane treatment of A/HeJ mice, which are particularly susceptible to the induction of lung adenomas, has been shown to cause impaired clearance of labeled tumor cells from the lungs.

Significance to Cancer Research: Recent findings with natural cell-mediated immunity may account for a significant portion of in vivo resistance to tumor growth, and may have important implications for immune surveillance.

Project Officer: Dr. Ronald S. Herberman
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 80 Funds: $218,962

CONTRACT RESEARCH SUMMARY

Title: Tumor Virus Expression in Normal and Transformed Cells

Principal Investigator: Dr. Kenneth Blackman
Name/Address Meloy Laboratories
Performing Organization: Rockville, MD

Contract Number: NO1-CB-74139
Starting Date: 6-15-77 Expiration Date: 6-14-81

Goal: To study expression of tumor viruses in tissue culture and in animals.

Approach: Viral biochemical, serologic, and biological parameters are monitored in different systems to define the regulatory mechanisms of tumor virus expression and to relate the expression to tumor production.

Progress: Using molecularly cloned Harvey Murine Sarcoma Virus DNA, the transforming region of the virus has been localized to the 5' end of the viral genome. The viral terminal repeat sequence enhances the efficiency of this transformation.

Using a probe specific for ecotropic env gene sequences, endogenous ecotropic murine leukemia virus (MuLV) genomes have been shown to be virtually complete linear copies in several mouse strains. The ecotropic viruses of these mice are highly related to each other by restriction endonuclease mapping.

A sub-genomic fragment from the 3' end of Friend-MuLV DNA, when rescued by a non-pathogenic helper virus, induces typical F-MuLV disease in susceptible mice.

A 69% sub-genomic fragment of molecularly cloned bovine papilloma virus DNA induces tumorigenic transformation of mouse cells in culture.

Significance for Cancer Research (NCP Objective 6 Approach 2)

Project Officer: Dr. Douglas R. Lowy
Program: Immunology Support
Technical Review Group: Ad Hoc Committee
FY 79 Funds: $85,350

CONTRACT RESEARCH SUMMARY

Title: National Cancer Institute Immunodiagnostic Reference Center

Principal Investigator: Dr. Angela Michaels
Performing Organization: Meloy Laboratories, Inc.
City and State: Springfield, VA

Contract Number: NO1-CB-63976
Starting Date: 7/11/75 Expiration Date: 7/10/81

Goal: To develop new assays for tumor-associated antigens, to use immunochemical techniques for the diagnosis of cancer, to evaluate humoral immune responsiveness of patients with cancer.

Approach: To use sensitive radioimmunoassays to quantitate human, monkey, and mouse alpha-fetoprotein and human chorionic gonadotropin in serum, to evaluate these assays both as diagnostic techniques for testicular, ovarian, and hepatocellular cancer, and as a means of monitoring the clinical response to therapy. Human lung tumors are being fractionated to isolate lung tumor specific antigen reactive with cancer and to establish a radioimmunoassay for this antigen. The contractor performed tests of humoral competence of patients with cancer or immunodeficiency diseases.

Progress: In a wide-ranging study of 1,000 patients with nonseminomatous germ cell tumor of the testis or hepatocellular tumors, alpha-fetoprotein and HCG assays have been shown to be of great value as an adjunct to diagnosis, in staging, and as a tool to monitor the chemotherapy of these tumors. These assays are being performed in collaboration with eight groups in monitoring the treatment of patients with hepatocellular or testicular germ cell tumors other than seminoma. In all cases in which the marker was elevated, but where there was no clinical evidence of disease, a positive marker always reflected occult residual tumor. A human lung tumor extract has been isolated and reacts with antiserum against human lung tumors but not against normal lung tissue. A radioimmunoassay has been established and is being used to detect very small quantities of this antigen in the serum of patients with lung tumors. This assay has been utilized in efforts directed towards establishing an immunodiagnostic test for human lung cancer and for monitoring the treatment of this disease. Humoral antibody assays have been established for ten antigens and these antibody assays are of critical importance in defining immune defects in patients with high incidence of neoplasia.

Significance to Cancer Research: These studies are helping to establish the usefulness of these multiple immunodiagnostic tests in the diagnosis of cancer, or as a means of monitoring patients during and after therapy.

Project Officer: Dr. Thomas A. Waldmann
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 80 Funds: $260,247

CONTRACT RESEARCH SUMMARY

Title: Immune Status of Patients Undergoing Immunotherapy

Principal Investigator: Dr. Douglas Strong
Performing Organization: Naval Medical Research Institute
City and State: Bethesda, MD

Contract Number: YO1-CB-00319
Starting Date: 3/1/80 Expiration Date: 9/30/80

Goal: To study the immune responses of cancer patients to tumor-associated antigens and to provide support for the Laboratory of Immunodiagnosis, NCI, for its detailed studies of cell-mediated immunity to human cancer.

Approach: Immunological tests to evaluate and monitor the immunocompetence of patients and their response to tumor-associated antigens include lymphocyte stimulation by mitogens and autologous tumor antigens; rosette assays for enumeration of T cells; leukocyte migration inhibition assays against recall antigens and tumor-associated antigens; and studies of direct cytotoxicity and antibody-dependent cell-mediated cytotoxicity against various human tumor cell lines.

Progress: This interagency agreement represents a continuation of work begun under Contract NO1-CB-63975. The various tests of cell mediated immunity (lymphocyte stimulation assays, rosette assays, leukocyte migration inhibition assays and cytotoxicity tests) have been established at the N.M.R.I. and the technical staff trained. The agreement has only recently been activated and although progress to date has been satisfactory, there is no data to report as yet.

Significance to Cancer Research: These studies will provide important information regarding the usefulness of various assays of cell-mediated immunity for immunodiagnosis and for monitoring the clinical course of cancer patients.

Project Officer: Dr. Ronald B. Herberman
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 80 Funds: $160,226

CONTRACT RESEARCH SUMARY

Title: Characterization of HLA Antigens of Donor's Lymphocytes by Serotyping and Cellular Typing

Principal Investigator: Dr. Rene J. Duquesnoy
Performing Organization: The Blood Center of Southeastern Wisconsin
City and State: Milwaukee, Wisconsin

Contract NO1-CB-04337
Starting Date: 6/2/80 Expiration date: 6/2/83

Goal: To analyze as carefully as possible the cell surface histocompatibility antigens of donors in order to analyze the relationship between these antigens and the ability of those donors' cells to mount appropriate immune responses.

Approach: Analysis of cell surface antigens is performed by two different detection systems: serology and cellular typing. The serologic analysis is performed using carefully screened alloantisera in assays of complement-dependent cytotoxicity. The cellular analysis is performed by analyzing the proliferative responses to homozygous typing cells (HTC typing) and by analyzing secondary restimulation of lymphocyte populations selectively immunized against alloantigens in primary response (PLT).

Progress: Since the contract is not to be activated until June 1980, no results are yet available. It is projected that by the end of FY 1980 approximately 70 donors will have been typed for HLA -A,B,C, and DR antigens by serologic tech niques, and approximately 15 donors will have been typed by cellular techniques.

Significance to Cancer Research: Considerable evidence from animal models and from epidemiologic studies in humans suggest that host cellular immune responses are crucial in determining the outcome of neoplastic diseases. Cellular immune responses are under control by genes in the major histocompatibility complex (HLA in man). In order to therapeutically manipulate these cellular immune responses, we must first understand their normal operation and genetic control.

Project Officer: Dr. J. Stephen Shaw
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 80 funds: $97,467

CONTRACT RESEARCH SUMMARY

Title: Computer Services

Principal Investigator: Mr. Francis A. McDonough
Name/Address Department of the Treasury
Performing 1435 G Street, N.W.
Organization: Washington, D.C. 20220

Contract Number: YO1-CB-90316
Starting Date: Nov. 15, 1979 Expiration Date: Sept. 30, 1980
Goal: To provide computer facility for mathematical computations related to biological systems modeling carried out in the Laboratory of Theoretical Biology and other groups in DCBD, NCI.

Approach: Facility is assessed through remote terminals

Progress: This facility is slowly being phased out as our own VAX computer is taking over most of our computations

Project Officer: Dr. Mones Berman
Program: Cancer Biology Support Site Visit Date: N/A
Technical Review Group: Ad Hoc
Relevance Review Group:
FY 80 Funds: $5,000.

CONTRACT RESEARCH SUMMARY

Title: A Screening System for Topical Chemotherapy of Mycosis Fungoides

Principal Investigator: Dr. Stanford Lamberg
Name/Address Johns Hopkins University
Performing Organization: Baltimore, MD

Contract Number: N01-CB-63927
Starting Date: 6-30-76 Expiration Date: Dec. 31, 1980

Goal: To apply chemotherapeutic agents developed by DCT for topical treatment of mycosis fungoides, a cutaneous lymphoma.

Approach: Chemotherapeutic agents are obtained commercially and from the DCT, NCI, and these agents are being screened by clinical patch testing in patients with mycosis fungoides.

Progress: Investigative new drug applications have been obtained from the FDA and patch test screening of these agents has begun at Hopkins and other affiliated institutions comprising the Mycosis Fungoides National Cooperative Group. More widespread testing of agents found to be active by patch test screening is in progress.

Significance for Cancer Research: (NCP Objective 6 Approach 2)

Project Officer: Dr. Gary Peck
Program: Biology Support
Technical Review Group: Ad Hoc Committee
FY 80 Funds: None

CONTRACT RESEARCH SUMMARY

Title: Radioimmunoassay of Immunoglobulin Molecules

Principal Investigator: Dr. James Harness
Performing Organization: Meloy Laboratories, Inc.
City and State: Springfield, Virginia

Contract Number: NO1-CB-63932
Starting Date: 3/14/80 Expiration Date: 3/13/81

Goal: To perform radioimmunoassays of immunoglobulin molecules IgM, IgA, IgG, IgD, and IgE in lymphocytes culture supernatants or in other biological fluids.

Approach: The contractor is to perform determinations of human IgG, IgA, IgM, IgD, and IgE, and mouse IgA and IgM by double antibody radioimmunoassay by procedures defined by the project officer and using reagents supplied by the project officer. This contract provides critically required research support for studies on the nature of immunodeficiencies that are associated with a high incidence of malignancy and on the cause of the immunodeficiency associated with malignancies of the B cell or plasma cell system. In addition, these studies are directed at defining retained immunological capabilities of T-cell leukemias.

Progress: The contract has established radioimmunoassays for IgG, IgA, IgM, and IgE of man and for IgA and IgM of the mouse. These assays have been used to quantitate the rate of immunoglobulin synthesis by pokeweed mitogen-stimulated, peripheral blood lymphocytes of man or of splenic lymphocytes of the mouse in in vitro cultures. Patients with a T-cell leukemia associated with the Sezary syndrome have been shown to have a malignant expansion of helper T cells whereas a patient with acute lymphocytic luekemia was shown to have a malignancy of the suppressor T cells. A subset of patients with common variable hypogammaglobulinemia has been shown to have excessive numbers of suppressor T cells that inhibit gamma globulin synthesis by B cells. Certain patients with post marrow transplant immunodeficiency have deficient B-cell function whereas others have excessive suppressor T-cell activity. Patients with multiple myeloma have hypogammglobulinemia due in part to excessive numbers of suppressor macrophages. These studies are defining the nature of disorders of the immune system that led to a high incidence of cancer. In addition, they provide information on the cause of immunodeficiency that arises secondary to certain forms of malignancy. Finally, these studies have provided insights into the retained functions of T-cell leukemias.

Significance to Cancer Research: These studies will help elucidate the status of the immune system during development of cancer.

Project Officer: Thomas A. Waldmann, M.D.
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Review Group
FY 80 Funds: $125,000

CONTRACT RESEARCH SUMMARY

Title: Computer Services

Principal Investigator: Mr. Martin Shaver
Name/Address Computer Services Division
Performing National Bureau of Standards
Organization: Gaithersburg, Maryland 20234

Contract Number: YO1-CB-80047
Starting Date: July 1, 1973 Expiration Date: September 30, 1980
Goal: To provide computer facility for mathematical computations related to biological systems modeling carried out in the Laboratory of Theoretical Biology and other groups in DCBD, NCI.

Approach: Facility is assessed through remote terminals.

Progress: This facility is slowly being phased out as our own VAX computer is taking over most of our computations.

Project Officer: Dr. Mones Berman
Program: Cancer Biology Support Site Visit Date: N/A
Technical Review Group: Ad Hoc
Relevance Review Group:
FY 80 Funds: $5,000

CONTRACT RESEARCH SUMMARY

Title: Small Animal Holding Facility

Principal Investigator: Andrew S. Tegeris
Performing Organization: Pharmacopathics Research Laboratories
City and State: Laurel, MD

Contract Number: NO1-CB-33887
Starting Date: 7/1/73 Expiration Date: 9/29/80

Goal: To maintain and observe animals on experiments designed to develop the means to reduce the effectiveness of external agents for producing cancer with a variety of types of materials, particularly mineral fibers and tobacco products.

Approach: Improve animal models of human cancer and evaluate effects of combined factors by assessing the response of the pleura of the rat to fibers and other forms of minerals that could be expected to reach this site in man.

Progress: Carcinogenic response to many types of durable fibers in the pleura of the rat were tested. Results suggest that fiber size is critical to carcinogenesis by asbestos and other fibers. Tobacco smoke fractions and air pollutant fractions are being evaluated for carcinogenicity on a quantitative level.

This is the terminal year.

Significance to Cancer Research; Animal models of human cancer are essential to identifying causative agents, mechanisms of action, and therapeutic trials.

Project Officer: Mearl F. Stanton, M.D.
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Relevance Review Group
FY 80 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: A Study of Phylogenetic Aspects of Neoplasia

Principal Investigator: Dr. John C. Harshbarger
Name/Address The Smithsonian Institution
Performing Organization: Registry of Tumors in Lower Animals
Washington, D.C.

Contract Number: NO1-CB-33874
Starting Date: 7/1/73 Expiration Date: 6/30/81

Goal: To collect, examine, classify, and preserve neoplasms in cold-blooded vertebrate and invertebrate animals, and to study experimentally the development of tumors in lower animals.

Approach: Specimens are acquired from personal field investigations or through submittal by other investigators. The specimens are examined grossly, histologically, and in some cases by electron microscopy. Diagnoses are established and the specimens are described. The world literature on tumors in lower animals is collected. Field investigations and experimental inductions of tumors in lower animals are carried out. The Registry serves in a consulting capacity to other agencies concerned with diseases in lower animals. A complete collection of the literature on neoplasms in ectothermic animals is maintained and kept current.

Progress: Specimen accessions during the past year numbered 179, bringing the total to 2219. The literature collection has increased to 3830 papers and a print-out of the abstracted data has been completed. Approximately half of the new accessions were confirmed to be neoplasms, while the remainder were parasitic, infectious, toxic, traumatic, or developmental diseases. Most of the neoplasms were in teleost fishes, amphibians, reptiles, and molluscs. In the invertebrate phyla, lesions that appear to be neoplastic by morphological criteria were found only in molluscs and arthropods (sects alone represented). Some 30 types of neoplasms have been recognized to occur at relatively high prevalences in certain species in certain locations. Types of tumors accessioned are listed in the 1979-80 Progress Report of the Registry. Foreign contributions came from Canada, England, Australia, India, Germany, Japan, and USSR. Seven original reports were published by the PI during the 1979 fiscal year.

Significance for Cancer Research: Field studies and anatomical studies indicate environmental carcinogens that may be of importance in human cancer epidemiology or may be useful in designing analytical experiments to determine mechanisms of tumorigenesis.

Project Officer: Clyde J. Dawe, M.D.
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Committee
FY 80 Funds: 180,200

CONTRACT RESEARCH SUMMARY

Title: Preparation and Analysis of Cell Surface Protein (CSP) Fractions

Principal Investigator: Dr. David Schlesinger
Performing Organization: University of Illinois
City and State: Chicago, IL

Contract Number: NO1-CB-74214
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To prepare and analyze the structure of cell surface protein.

Approach: The C-terminal fragment of CSP (fibronectin) is prepared by pepsin digestion, two-dimensional electrophoresis or column chromatography, and high pressure liquid chromatography. The heparin- and collagen-binding domains of CSP are prepared by affinity chromatography. The fragments are sequenced by sequenator or micro-sequencing protocols.

Progress: Two C-terminal peptide fragments have been isolated and analyzed. Purified heparin- and collagen-binding fragments have also been studied. Each fragment has been found to have unique and distinctive amino acid compositions and N-terminal sequences. Further sequencing and analysis of other biologically active fragments should provide insight into the mechanisms of CSP's multiple biological effects. Dr. Schlesinger will continue to characterize the structure of these various fragments of CSP.

Significance to Cancer Research: CSP (fibronectin) is a major adhesive glycoprotein that is present in a variety of normal connective tissue and epithelial cell types and is absent from many types of cancer cells, especially metastatic cells. Knowledge of the structure of CSP will further the understanding of the cancer process.

Project Officer: Dr. Ira Pastan
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Committee
FY 80 Funds: $46,000

CONTRACT RESEARCH SUMMARY

Title: Computer Services

Principal Investigator: Mr. Wayne H. Anderson
Name/Address Commander USAARDC
Performing ATTN: DRDAR-MSE-T (Mr. W.H. Anderson)
Organization: Aberdeen Proving Ground, MD 21010

Contract Number: YO1-CB-80315
Starting Date: May 1, 1978 Expiration Date: Sept. 30, 1980
Goal: To provide computer facility for mathematical computations related to biological systems modeling carried out in the Laboratory of Theoretical Biology and other groups in DCBD, NCI.

Approach: Facility is assessed through remote terminals.

Progress: This facility is slowly being phased out as our own VAX computer is taking over most of our computations.

Project Officer: Dr. Mones Berman
Program: Cancer Biology Support Site Visit Date: N/A
Technical Review Group:
Relevance Review Group:
FY 80 Funds: $2,000.

CONTRACT RESEARCH SUMMARY

Title: Preparation of Purified Wheat Proteins and Wheat Protein Fractions

Principal Investigator: Dr. Donald Kasarda
Performing Organization: U.S. Department of Agriculture
City and State: Berkeley, California

Contract Number: Y01-CB-60312
Starting Date: 10/1/79 Expiration Date: 9/30/80

Goal: To obtain chemically defined fraction of wheat gliadin for use in studies of gluten-sensitive enteropathy (coeliac sprue).

Approach: Wheat gluten will be chemically fractionated and subjected to cyanogen bromide cleavage. Homogeneous fragments of gliadin, as well as gliadin itself, will then be supplied.

Progress: The contractor has supplied alpha-gliadin according to the contract schedule. The alpha-gliadin has been used in in vitro studies of responses of lymphoid cells to defined gliadin preparations -- cells obtained from both patients and normals. Finally, alpha-gliadin has been used in a radiometric assay of anti-gliadin production by lympoid cells in vitro which will ultimately enable us to study the regulation of anti-gliadin responses in vitro.

Significance to Cancer Research: Gluten-sensitive enteropathy is a disease associated with a high incidence of malignancy. Elucidation of the pathogenesis of gluten-sensitive enteropathy will provide insight into the factors which lead to the onset of malignant disease.

Project Officer: Warren Strober, M.D.
Program: Cancer Biology Support
Technical Review Group: Breast Cancer Task Force Committee
FY 80 Funds: $18,600

LIBRARY
NIH
Amazing Research.
Amazing Help.

http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

NIH LIBRARY

NIH LIBRARY
3 1496 00185 6783